A

LABORATORY SYLLABUS

OF

CLINICAL PATHOLOGY

BY

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PREFACE.

Every teacher of clinical pathology, no doubt, has observed, with alarm, the rapid growth in volume of the subject-matter which he is obliged to take up in his classes, while the number of hours that are placed at his disposal for this purpose is remaining practically the same. Not many years ago the course in clinical microscopy, as it was then and is still at times wrongly called, was largely a course in what the name then suggested, viz., a course in the microscopic examination of the blood and the various secretions and excretions of the body, coupled with a relatively meager review of some of the simpler methods of chemical analysis of the same material.

Nowadays a course along these older lines would no longer be deemed sufficient to familiarize the student with those methods of laboratory investigation which are in daily use in any up-to-date clinical laboratory, and a knowledge of which is rightly regarded as essential by a board of modern medical examiners.

At the University of Maryland the class in clinical pathology is divided into two sections, the instruction of each of which extends over a period of fifty-eight afternoons, of two hours each, with thirteen additional single-hour periods. This covers the time spent in the laboratory proper, in lectures, demonstrations and recitations. Our principal difficulty in the past has been to provide adequate time for actual laboratory work and recitations. During the laboratory periods a certain amount of time has, in the past, been spent in explaining the work to be done, thus leaving not more than one hour and a half for actual work. The recitation work, of course, has suffered the most, and gradually has become a negligible factor; as a consequence systematic home reading is practically not done at all.

Various measures have suggested themselves as remedies for this undesirable state of affairs, the simplest evidently being an

increase in the number of teaching hours. But until a general readjustment of the curriculum, of the third and fourth years more particularly, is adopted by all the medical schools of our country, this course is as yet not available.

Meanwhile, it has occurred to me, that a considerable amount of time might be gained by eliminating the brief talks at the beginning of each lesson, which after all do not cover the ground as fully as is actually desirable, and by substituting therefor a set of printed instructions which would serve as a guide to the afternoon's work. With this idea in mind I have written this little syllabus. It will be noted that the subject-matter has been arranged in connection with each lesson under three headings, viz.: (1) instructions to the assistants regarding the nature of the material, the reagents and apparatus that are required for each lesson; (2) instructions to the student for the afternoon's work, with directions as to the manner in which his findings and results are to be reported; (3) a set of questions based on the work done in the laboratory and upon home reading, which are to be answered in writing at home and the answers presented to the instructor as a part of the work done.

The plan then is, from time to time, to call in the books in which these data are assembled, for criticism and correction. This involves a good deal of work on the part of the instructor, to be sure, which cannot be avoided, however, and will serve to keep him in touch with the status and the progress made by the individual student, which, after all, is as it should be.

It will be noted that the subject-matter to be covered in the laboratory has been arranged on the basis of thirty-nine lessons, each lesson to occupy a two-hour period. This I am inclined to regard as the minimum of time that should be available for actual practical work. Where more time is at the instructor's disposal some of the lessons may, of course, be advantageously repeated, but I dare say that the majority of teachers will not be able to devote many more hours to work in the laboratory proper, as they will require at least eighteen hours for lectures and as many for recitations. The table of contents will give an idea of the work that is to be covered in the laboratory.

In conclusion, I would draw attention to the heading "Read-

ing" at the end of each lesson. I have left this blank so that any instructor who does not use my own text-book in his course may give the page references to his students in those works which happen to be his favorites.

Trusting that this little book may be found serviceable by the student and the instructor alike, and that the latter by its use may gain more time for his lectures and demonstrations than was at his command heretofore, I submit it to both with the hope that it may be accorded the same generous reception that my *Clinical Diagnosis* has met with throughout its many editions.

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LABORATORY SYLLABUS OF CLINICAL PATHOLOGY.

Lesson 1.—*Apparatus and Reagents Required.*—Slides and cover-glasses (No. 1) that have been cleansed with soap and water; sterilized gauze sponges in pairs, wrapped in muslin; Hagedorn needles; individual bottles of alcohol (2 oz.).

Cleanse the lobe of the ear of your working partner with alcohol; dry; puncture with needle that has previously been placed in alcohol for a couple of minutes and dried; wipe away first drop; mount next drop (small size) on a cover-glass and invert immediately on a slide; the drop should spread out by capillary attraction; if this does not occur, make another mount. Examine with oil-immersion lens.

Note and draw appearances of red cells where they are well separated.

Answer the Following Questions in Writing.—What is the form and color of the normal red cell? what is its size? what are the normal variations in size? what does the central pale area denote? what is meant by the following terms: normocyte, microcyte, macrocyte, microcytosis, macrocytosis, anisocytosis, poikilocyte, poikilocytosis.

Note and draw crenated red cells; cells in money-roll formation. Note and draw the various types of leukocytes that occur in normal blood, with the exception of the so-called mast cell, viz., the small mononuclear, the large mononuclear, the finely granular polymorphonuclear and the coarsely granular polymorphonuclear, indicating size and form of nucleus, the relation in bulk between nucleus and cytoplasm and the presence or absence and size of granules.

Draw leukocytes undergoing ameboid movements.

Answer the Following Questions in Writing.—What is the size (*a*) of a small, and of (*b*) a large mononuclear leukocyte cell, (*c*) of a finely granular and (*d*) of a coarsely granular polymorphonuclear leukocyte as compared with a red cell. What type is the most common? what next in order? which next and which last? which are capable of undergoing ameboid movements? which are capable

of phagocytic activity? which type after emigration from the bloodvessels constitutes the common pus corpuscle?

Reading.—

Lesson 2.—*Apparatus and Reagents Required.*—Hemocytometers; normal salt solution, 3 per cent. acetic acid and alcohol in individual bottles (2 oz.); Hagedorn needles; sterilized gauze sponges in pairs, wrapped in muslin.

Examine and draw (*a*) the red pipette, permitting a dilution of 1 to 100 and 1 to 200; (*b*) the white pipette, giving a dilution of 1 to 10 and 1 to 20, and (*c*) the counting slide—the latter in profile with the cover-glass adjusted over the central ruled area. Examine the ruling with the low power (objective 3 L. or $\frac{2}{3}$ B. & L.) and draw it.

Answer the Following Questions in Writing.—What diluting fluid do you use (*a*) when making red counts? (*b*) when making white counts? What degree of dilution do you commonly use (*a*) when making red counts? (*b*) when making white counts? why?

Using Simon's counting chamber, what is the size of a small square? what of a rectangle? what of a large square? what are the corresponding cubic contents of a mounted drop? In which squares do you count the red cells and in which the white?

Experiment.—(*a*) Puncture the ear of your working partner, as described in Lesson 1; draw blood into your red pipette to the mark 0.5 and saline to the mark 101, giving a dilution of 1 to 200 in the bulb; detach the rubber tubing and shake for several minutes; blow out the diluting fluid in the stem of the instrument and mount a drop of the contents of the bulb on your counting surface; adjust the cover-glass; slide this to and fro until Newton's colored rings appear; let stand for a few minutes and now count the red cells in 100 small squares, using the low power of the microscope, marking them down in the diagram, that you have drawn, with a colored pencil; those on the top and left lines are counted in and those on the right and bottom lines out. Determine the average per one small square and multiply by the corresponding cubic contents and the degree of the dilution. The resultant figure indicates the number of red cells in 1 c.mm. of blood.

(*b*) Charge the white pipette in a similar manner to the mark 0.5; dilute to the mark 11 with 3 per cent. acetic acid and shake for several minutes; blow out the diluting fluid from the stem of the instrument; mount a drop of the diluted blood on the counting

surface; adjust the cover-glass; let stand for a few minutes, then count the leukocytes contained in 100 of the large squares, using first the middle power of the microscope and repeating with the low power, marking down the cells in the diagram that you have drawn. Determine the average for a single large square; multiply by the corresponding cubic contents and the degree of dilution. The resultant figure indicates the number of leukocytes in 1 c.mm. of blood.

Answer the Following Questions in Writing.—Why does the mixture of blood and acetic acid in the bulb of your mixing pipette turn brown? what is formed?

What is the normal number of (*a*) the red cells, and (*b*) of the leukocytes per c.mm. of blood. What is meant by the following terms: oligocythemia, polycythemia, polyglobulism? What is the difference between relative and absolute polycythemia? Name conditions under which the former and the latter may be encountered. How high and how low may the red count go in disease. What is meant by the following terms: leukocytosis, hypoleukocytosis, leukopenia, hyperleukocytosis. How high and how low may the leukocyte count go in disease?

Reading.—

Lesson 3.—*Apparatus and Reagents Required.*—Talquist's color scale; Sahli's hemoglobinometer; v. Fleischl's hemometer; medicine droppers; distilled water; normal salt solution; decinormal hydrochloric acid and alcohol in individual bottles (2 oz.); Hagedorn needles; sterilized gauze sponges in pairs wrapped in muslin.

1. Make hemoglobin estimations according to the three methods indicated and compare results, using the blood of your working partner. (*a*) Using Talquist's color scale, mount a drop of blood on a piece of the accompanying filter paper and compare the color while still wet with the color scale of the Talquist booklet; note the result.

(*b*) In using Sahli's hemoglobinometer, place a little decinormal hydrochloric acid in the graduated test-tube, say up to the 20 mark. Fill the blood pipette up to the 20 c.mm. mark with blood, in solid column, and gently blow this out into the decinormal acid; shake until the diluted blood has turned brown and is perfectly transparent; add water, a couple of drops at a time, and shake after each addition until the color of the mixture when placed in the accompanying little stand, alongside the standard solution, matches that of the

latter; the figure corresponding to the level reached indicates the percentage of hemoglobin.

(*c*) Using the v. Fleischl instrument, half-fill each chamber of the well that fits the central aperture of the stage of the instrument with water. Puncture the ear or finger and allow one of the capillary pipettes that accompany the apparatus to fill itself by merely holding one end in contact with the drop. Transfer this volume of blood to one of the chambers in which water has just been placed and agitate the pipette gently until all blood has diffused out. Wash through with a few drops of water; fill both chambers with water until a convex meniscus is formed at the top of each. Adjust the colored wedge under the stage of the instrument; turn the well so that the half, filled with water only, stands above the wedge; by means of the rack-and-pinion adjustment, turn the latter to and fro until the colors in the two chambers match; this must be done in a darkened room, using artificial light; the colors are best compared by viewing them through a paper tube, so as to exclude all outside light. The figure opposite the little notch in the scale well, on the stage of the instrument, indicates the percentage of hemoglobin.

2. Do a red count in the same person whose hemoglobin has just been studied, as described in Lesson 2. Determine the percentage of the normal that the count represents, taking 5,000,000 as average normal, and hence as representing 100 per cent.

3. Determine the color index for the individual examined by dividing the percentage of hemoglobin by the percentage of red cells.

Answer the Following Questions in Writing.—What is the normal percentage of hemoglobin, gravimetrically expressed? What relation exists between a 100 per cent. reading obtained by means of a hemoglobinometer and the actual amount of hemoglobin? To what percentage, gravimetrically, would a reading of say 65 per cent. correspond?

What is meant by the term color index? what is it normally? what does an increased index usually indicate? what is the index in chlorosis? what in the secondary anemias? What is meant by oligochromemia? hypocytochromia? hypercytochromia? What is the supposed explanation of the latter?

Reading.—

Lesson 4.—*Apparatus and Reagents Required.*—Clean slides; Hagedorn needles; gauze sponges in pairs, wrapped in muslin; alcohol; (*a*) a 1 per cent. aqueous solution of eosin; (*b*) a 1 per cent.

aqueous solution of methylene blue; Jenner's stain; Wright's stain —all reagents in individual bottles; staining trays; distilled water in 100 c.c. lots for every six men; tumblers or wash bottles for washing with tap water; blotting or filter paper for individual use.

Cleanse needle and lobe of the ear with alcohol; puncture; mount a moderate-sized drop of blood near the end of one slide; bring the narrow edge of a second slide in contact with the blood and let the drop spread along the edge by capillary attraction; then, without using any pressure, sweep the edge of the second slide along the entire surface of the first, starting at an acute angle, rapidly coming to a right angle and ending at an obtuse angle. The resultant smear will appear in ridges. Make half a dozen smears and let dry in the air. These will keep in good condition for staining for at least a week when wrapped in paper. Place two smears on the bars of the staining tray; cover with alcohol to fix and leave for five minutes; wash off with tap water; cover one with eosin solution (acid dye) and the other with methylene-blue solution (basic dye); stain for one minute; wash off; allow to dry in the air or blot with filter paper. Stain a third specimen, without preliminary fixation, for three or four minutes with Jenner's stain (a neutral mixture of eosin and methylene blue), by flooding the slide with the stain; wash off with tap water and allow to dry in the air. Stain a fourth specimen with Wright's stain (a neutral mixture of eosin and methylene azure) as follows: Flood the slide on the bars of the staining tray with Wright's stain. After forty-five seconds add an equivalent volume of distilled water; leave the mixture for four minutes; wash off the stain with tap water and dry the specimen by blotting.

Examine the eosin specimen first. Note (*a*) that the red cells have taken the eosin stain intensely, (*b*) that the nuclei of all types of leukocytes are scarcely stained at all, or at most a faint pinkish—action upon the achromatic substance of the nuclei; (*c*) that the cytoplasm of the small and large mononuclear leukocytes is likewise nearly colorless or a faint pink—action upon the paraplasmic component of the cytoplasm; (*d*) that the cytoplasm of the finely granular polymorphonuclear leukocytes stains a more definite pinkish color while the granules are colorless; (*e*) that the granules of the coarsely granular polymorphonuclear leukocytes are stained a bright red.

Material that stains with eosin is said to be eosinophilic or oxyphilic.

Illustrate the above points, using red pencils, and note the degree of oxyphilia by the mark +++, = and o.

Examine the methylene-blue specimen next: Note (*a*) that the nuclei of all the different types of leukocytes have taken the basic methylene-blue stain; they are hence said to be basophilic. The same is true of the cytoplasm—spongioplasm—of the small and large mononuclear leukocytes and to a slight extent of the spongioplasm of the eosinophilic—coarsely granular leukocytes. The granules of the finely and coarsely granular leukocytes remain colorless, but if by any chance a so-called mast-cell be encountered, it will be noted that its granules are deeply colored by the basic dye. The red cells hardly take the stain at all—the slight staining is probably due to the presence of traces of the original spongioplasm of the cell.

Illustrate the above points, using a blue pencil for all basophilic elements, and note the degree of basophilia by the marks +++, ++, + and 0.

Next examine the Jenner—eosinate of methylene-blue—specimen. Note that the red cells take a reddish-brownish tone, due to the marked affinity for the acid stain on the part of the hemoglobin and the slight affinity for the basic stain on the part of the basophilic component of the cell. All cell nuclei are colored blue, as is also the cyto-spongioplasm of the small and large mononuclear leukocytes; to a less extent that of the eosinophiles, while the granules of the mast cells are deeply colored by the methylene-blue; the eosinophilic granules appear a bright red and the fine granules of the common polymorphonuclear leukocytes, which took neither the acid nor the basic stain, now are colored a fine purplish red; they are hence said to be neutrophilic. The blood platelets which may be seen here and there among the red cells, or coming from their interior through a cleft, are colored light blue.

Illustrate all these appearances, using colored pencils, and note which elements are oxyphilic, basophilic, and neutrophilic.

Finally, examine the Wright specimen. Note the brilliant color of the cell nuclei—reddish purple—which is due to the methylene azure; in some of the large and small mononuclears isolated granules will be seen, colored reddish purple, the so-called azurophilic granules; the neutrophilic granules are not as well stained as in the Jenner specimen; the eosinophilic granules appear a brownish red, but are not as striking as in the Jenner preparation; the basophilic mast-cell granules appear a reddish purple; the cytoplasm of the small and large mononuclears is practically colorless; the blood-platelets appear a bluish gray, with a reddish-purple central portion.

Illustrate the various appearances, using colored pencils, and note which elements are azurophilic.

Answer the Following Questions in Writing.—Which chemical group in the make-up of the aniline dye determines whether the product has (*a*) acid or (*b*) basic properties? What is meant by a neutral dye mixture? Name an acid dye, a basic dye, two neutral mixtures? What is the salt-forming group of a dye called? What is meant by the chromogenic group of a dye? Give an example of a chromogenic group. What is meant by the terms oxyphilic, eosinophilic, neutrophilic, basophilic, azurophilic, orthochromatic, metachromatic.

Which is the essential basic constituent of all the so-called Romanowsky mixtures? name some of these mixtures. What relationship exists between methylene-blue and methylene azure? What is a polychrome or panoptic stain?

Why is it unnecessary to fix with alcohol before using Jenner's or Wright's stain?

What volumetric relation exists between nucleus and cytoplasm (*a*) in the small, (*b*) in the large mononuclear leukocyte? Does this rule hold for all cells of this type irrespective of their age? What is a "transition form," (*a*) morphologically considered, (*b*) in its relation to the large mononuclear leukocyte?

What is meant by a polynuclear cell, as contrasted with a polymorphonuclear cell?

What are the essential morphological points of difference between the neutrophilic and eosinophilic leukocytes of normal blood.

Reading.—

Lesson 5.—*Apparatus and Reagents Required.*—Clean slides; sterile gauze sponges, in pairs, covered with muslin; Hagedorn needles; alcohol; Jenner's stain, Wright's stain in individual bottles (2 oz.); staining trays; distilled water; wash bottles; filter paper.

Students should also be furnished with stained blood smears from (*a*) a typhoid case about the end of the first week; (*b*) a typhoid case from the third week; (*c*) a typhoid convalescent whose temperature has been normal for several days.

1. Prepare blood films from your working partner and stain with Jenner's stain, as described in Lesson 4. Wipe the reverse side of the slide dry and examine while the blood side is still wet, using the lowest power of the microscope. (Leitz obj. 3, ocular 4). Note (*a*) the size, the color and general distribution of the red cells; (*b*) the

leukocytes among the red cells which appear as little blue dots; with practice the different types may readily be differentiated from one another; the small mononuclears appear as purely blue dots, the size of a red cell; the large mononuclears are a lighter blue and much larger than a red cell; the polynuclear neutrophiles show a lobed blue nucleus lying in a slightly pinkish cell body; the eosinophiles show a lobed blue nucleus lying in a bright red cell body; in the basophiles the granules appear almost black, scattered over the surface of the cell body, while the nucleus appears light blue.

Try to differentiate the various types in every blood smear that you examine until you feel as confident of your ability to do so with the low power as with the oil-immersion lens. While making your low-power survey, get in the habit of noting the number of leukocytes in a field. In the course of time you will learn to estimate their absolute number by simple inspection. If your slide becomes dry while working with the low power wet it again with water. After having finished your low-power survey, allow the slide to become air-dry and then examine with the oil-immersion lens. The best portion of the slide is the first third, counting from the point where the drop of blood was first mounted. Move the slide up and down, and, following the ridges of the smear, note in tabular form all the leukocytes that you encounter, in sets of five, as shown below; count an even 300 and calculate the percentages of the different types.

Small mononuclears:	𝍸 𝍸 𝍸 𝍸 𝍸, etc. . .	80
Large mononuclears:	𝍸 𝍸 𝍸	15
Polynuclear neutrophiles:	𝍸 𝍸 𝍸 𝍸 𝍸, etc. . .	198
Polynuclear eosinophiles:	𝍸 /	6
Polynuclear basophiles:	/	1
		300

Having familiarized yourself with the different types of cells, practice counting a large number, in your mind, before putting them down, and then note them as follows:

S.M.	L.M.	P.M.	P.E.	P.B.	
12	4	52	1	0	
26	7	40	2	0	
20	8	46	1	0	
20	7	50	2	2	
78	26	188	6	2	= 300

Small mononuclears	=	26.0 per cent.
Large mononuclears	=	8.6 "
Polynuclear neutrophiles	=	62.6 "
Polynuclear eosinophiles	=	2.0 "
Polynuclear basophiles	=	0.6 "

A count of this order is termed a differential count.

Now do a so-called Arneth count: To this end classify a hundred neutrophiles according to the number of *separate* nuclear lobes, arranging them in five groups.

Study in the same manner the blood smears from a typhoid patient obtained (*a*) at the end of the first week; (*b*) in the course of the third week of the disease; (*c*) during the first week of convalescence. Arrange your data in tabular form under the headings: (*a*) Differential count; (*b*) Arneth count.

Answer the Following Questions in Writing.—What are the normal percentages of the different leukocytes (*a*) in the adult; (*b*) in children younger than six years? What is meant by lymphocytosis, splenocytosis, neutrophilic hyperleukocytosis, neutrophilic hypoleukocytosis, eosinophilia, hypereosinophilia, hypoeosinophilia, basophilia? What is meant by Simon's septic factor?

Name some pathological conditions (*a*) in which the neutrophilic elements are increased; (*b*) in which they are diminished; (*c*) in which the eosinophiles are increased; (*d*) in which they are diminished; (*e*) in which the small mononuclears are increased; (*f*) in which the large mononuclears are increased.

Supposing the total count in a given case was 2500 and the differential count showed 50 per cent. of small mononuclears and 40 per per cent. of polynuclear neutrophiles, which factor would you regard as the essential one—the decrease of the polys or the increase of the small monos; calculate the corresponding absolute values per cubic millimeter, and compare them with the normal.

Reading.—

Lesson 6.—Students to be furnished with stained blood smears from the following pathological conditions: (*a*) acute appendicitis, (*b*) pneumococcus pneumonia; (*c*) meningococcus meningitis; (*d*) streptococcus endocarditis; (*e*) an acute exacerbation of a pulmonary tuberculosis; (*f*) tubercular osteomyelitis. Use Jenner's solution for staining purposes.

Examine the specimens first with the low-power and then with the oil-immersion lens. Note with the low-power whether the total number of the leukocytes appears to be increased, and the extent, as compared with a slide of normal blood; then do a differential count, paying particular attention to the presence or absence of eosinophiles; compare the findings in the first four cases with those in the last two. Give your findings in the form of a formal report.

Answer the Following Questions in Writing.—In infections with what organisms do you find an increase of the neutrophiles associated with a decrease or absence of eosinophiles, and in which do you find an increase of the neutrophiles associated with normal or even increased percentage values of the eosinophiles? What do you mean by an epicritic eosinophilia?

Lesson 7.—Students to be supplied with stained smears from (*a*) a case of whooping-cough; (*b*) a quiescent pulmonary tuberculosis, (*c*) a case of tapeworm infection, (*d*) a case of chronic lymphatic leukemia, (*e*) a case of hookworm infection, (*f*) from a chronic malarial infection. Study these various slides as in Lesson 6, (*a*) with a low power, noting the total number of the leukocytes as compared with the normal; (*b*) do a differential count in each. What are the salient features in each case?

Reading.—

Lesson 8.—Students to be supplied with blood smears (*a*) from a case of acute lymphatic leukemia, stained according to Wright's method; (*b*) from a case of chronic lymphatic leukemia, stained according to Wright; (*c*) from a case of chronic myelocytic leukemia, stained with Jenner's stain.

With the low power note the total number of leukocytes, as compared with the normal, and gauge the extent of the increase in actual numbers. Examine the first smear with the oil-immersion and carefully compare the structure of the predominating cell and its nucleus with that controlling the picture in the second case. Make drawings illustrating the size of these cells, as compared with the size of a red cell, and of the arrangement of the chromatin in the nucleus. The predominating cell in the first specimen is the so-called lymphoidocyte; in addition cells will be seen which correspond to the description of the leukoblast; search also for Rieder forms, *i. e.*, cells of the types mentioned in which the nucleus has become polymorphous. Incidentally, note the pallor of the red cells and the occurrence of nucleated red cells—almost exclusively normoblasts. Make a differential count in both (*a*) and (*b*); while doing so note the number of nucleated red cells that you encounter while counting 300 leukocytes. While differentiating the lymphoidocytes in specimen (*a*) group them into two categories, viz., those which are distinctly larger than a red cell, macrolymphoidocytes, and those which correspond to the small monos in size, microlymphoidocytes. Can you find any true small monos (lymphocytes) in the smear? Now examine the specimen from the case of chronic myelocytic leukemia.

Note the following points: (*a*) the enormous increase in the total number of the leukocytes—gauge it; (*b*) that nearly all the leukocytic elements are granule-bearing; (*c*) that many of the neutrophilic and eosinophilic polymorphs are smaller than normal; (*d*) that many neutrophilic and eosinophilic elements are mononuclear—myelocytes; (*e*) that neutrophilic and eosinophilic myelocytes are of two types—a large type (macromyelocyte), and a small type (micromyelocyte); (*f*) that the granules of the eosinophilic myelocytes are of a bluish-purple color, whereas the eosinophilic polymorphs have bright red granules; (*g*) that many of the mononuclear neutrophilic cells have a bean-shaped nucleus—the metamyelocytes. Further note the occurrence (*h*) of large mononuclear leukocytes, devoid of neutrophilic, eosinophilic and mast-cell granules, but presenting a well-staining basophilic spongioplasm—leukoblasts; (*i*) of cells showing the general structure of a small mononuclear leukocyte, but of larger size and often showing the cytoplasm gathered in knobs on the periphery—macrolymphocytes; stained with an azure mixture these could be shown to be lymphoidocytes; (*j*) true small monos and large monos are very scarce; (*k*) polymorphonuclear basophiles—mast cells—and corresponding mononuclear forms are very common—some with fine granules, others with coarse granules. Nucleated red cells, mostly normoblasts, some undergoing mitosis, will also be met with.

Illustrate the Above Points.—Attempt a differential count of 200 cells.

Answer the Following Questions in Writing.—What is a lymphoidocyte, a leukoblast and a myelocyte, (*a*) structurally considered, (*b*) ontogenetically considered? Draw a schema, showing the interrelationship between the different kinds of leukocytes themselves and the red cells. Where do the lymphocytes originate? where the granulocytes? In what manner does the blood picture of the ordinary lymphocytosis differ from that seen in acute lymphatic leukemia, so-called? In what manner does hyperleukocytosis of the septic type differ from that seen in chronic myelogenous leukemia? show the points of difference in a tabular form. Does the appearance of myelocytes *per se* warrant the diagnosis of myelogenous leukemia; if not, why not? What is meant by myelocytosis or myelemia?

Reading.—

Lesson 9.—Students to be supplied with blood smears from (*a*) a case of chlorosis; (*b*) a case of secondary anemia, due to a severe

streptococcus infection; (*c*) a case of secondary anemia due to cancer of the stomach; (*d*) a case of pernicious anemia (Biermer type), all stained with Jenner's stain. Study the smears with the oil-immersion lens, in reference to the following points, arranging your findings in tabular form: (*a*) The amount of coloring matter in the individual cell—whether there is normocytochromia, hypocytochromia, or hypercytochromia; to this end pay attention only to cells that have not been flattened out, but show the central pale area; (*b*) abnormal variation in size—anisocytosis and its extent; (*c*) abnormal variation in form—poikilocytosis and its extent; (*d*) tendency to oversize (macrocytosis); (*e*) tendency to oval form; (*f*) the occurrence of polychromatophilic red cells—their relative number; (*g*) the occurrence of stipple cells—cells undergoing so-called granular degeneration—their relative number; (*h*) the occurrence of normoblasts; (*i*) of megaloblasts; (*j*) the number of blood platelets, whether increased or diminished. *Draw all These Features.*

Now examine the smears with the low power and note whether the leukocytes are present in normal numbers or whether they are increased or diminished. *In fine* do a differential leukocyte count.

Answer the Following Questions in Writing.—What is the color index (*a*) in chlorosis; (*b*) in secondary anemia; (*c*) in pernicious anemia? What is meant by (*a*) hypocytochromia; (*b*) hypercytochromia? What is meant by (*a*) anisocytosis, (*b*) poikilocytosis; (*c*) macrocytosis; (*d*) polychromatophilia or polychromasia; (*e*) an erythroblast? What does polychromatophilia indicate? is it a regenerative or a degenerative symptom? (*f*) What is a stipple cell? is it a regenerative or a degenerative symptom? Under what pathological conditions are stipple cells notably met with? What are the cardinal features (*a*) of a normoblast? (*b*) of a megaloblast? What conclusion may be drawn from the presence of erythroblasts in the circulating blood? What is the prognostic significance of the megaloblast as compared with the normoblast? What relation does the megaloblast bear to the normoblast? from what cell is it derived? What is meant by a blood platelet? what is the origin of the platelets? under what conditions are they notably increased? in what type of anemia are they notably diminished in number? In distinguishing between Biermer's anemia and the pernicious type of anemia seen in cancer cases, other things being equal, in what direction would leukopenia point? which cells would be deficient and which relatively increased?

Reading.—

Lesson 10.—*Apparatus and Reagents Required.*—Hagedorn needles; sterilized gauze sponges in pairs, covered with muslin; small test-tube racks for agglutination tubes—one for every man; a dozen agglutination tubes per student, made from glass-tubing one-fourth inch inside diameter, each two inches long (these can be prepared by the student himself); clean slides and cover-glasses; vaselin in tubes—one for every two men; alcohol in individual bottles; capillary pipettes—these also can be prepared by the students themselves; rubber nipples to fit the capillary pipettes; Dunham solution cultures—not more than twenty-four hours old, grown at room temperature, of the typhoid bacillus, of the paratyphoid A and paratyphoid B—a culture of each for every six men; corresponding antisera, diluted so as to give a definite agglutination reaction in one hour, at room temperature, when the microscopic method is applied—10 c.c. for every six men; a high-titer serum against each one of the organisms mentioned—in such dilution as to give a macroscopic reaction in five minutes—3 c.c. for every six men. A bichloride or lysol basin to be provided for every twelve men (*a*) for purposes of hand disinfection, (*b*) for disinfecting apparatus used at the end of the lesson; glass pencils—one for each student. Have Bunsen burners available.

Directions to the Student.—Charge agglutination tubes with about 1 c.c. of the three cultures given out and label correspondingly; charge another set of tubes each with 1 c.c. of the corresponding antisera—label, noting the degree of dilution; charge a further set of tubes with about 0.5 c.c.—8 drops—of the three high-titer sera—label, noting degree of dilution. Have a separate pipette for each tube, marked with glass pencil.

Experiment A.—*Macroscopic Slide Agglutination Method.*—On a slide mount, in a row, a large drop from each one of the cultures marked, T, PTa and PTb; place two similar rows of drops of the same organisms below these. To each drop of the top row now add one drop of the high-titer antityphoid serum; to each one of the second row add one drop of the high-titer antiparatyphoid-A serum and to each drop of the third row one drop of the high-titer antiparatyphoid-B serum. Place the slides on the stage of the microscope and view them by looking at the mixtures, with the naked eye, on a slant, and then with the low power of the microscope. Clumps will begin to form in some of the drop mixtures within a few minutes; note that this will occur first and become most marked in the homologous mixtures, viz., in the typhoid-anti-

typhoid drop, in the p-typhoid-a-anti-p-typhoid-a and in the para-typhoid-b-anti-p-typhoid-b drops, while in the crossed mixtures clumping will either not occur at all or develop much later and then be less marked. In this manner it is evident that the nature of an unknown antiserum or an unknown bacillus may be identified if the corresponding reagent is available.

Draw these appearances and append explanatory legends.

EXPERIMENT B.—*Microscopic Slide Agglutination Method.*—Prepare two vaselin rings on each one of three slides; the walls should be of uniform height—one-eighth inch; leave no gaps; label slide (1) T; slide (2) PTa; slide (3) PTb. Charge each vaselin chamber of slide 1 with a drop of the typhoid culture; add to one a drop of the antityphoid serum, of moderate titer, and to the other a drop of saline. Adjust a cover-glass, so as to be everywhere in contact with the vaselin wall; press the cover down evenly and gently until it touches the drop mixture below. Prepare the other two slides in a similar manner with the corresponding organisms and antisera. Examine the mixture with the low power of the microscope with the condenser out, using the flat mirror and a subdued light; the saline-bacillary drops serve as a check on the corresponding bacillary antisera mixtures, to indicate whether the bacillary emulsions were homogeneous to begin with, and to call attention to the occurrence of auto-agglutination, which, of course, would invalidate the significance of an agglutination reaction in the corresponding bacillary antiserum specimen. With the low power of the microscope the focussed drop mixtures will appear evenly granular. Set the specimens aside and reëxamine at intervals of ten minutes; make your final readings at the end of one hour. You will then note that the background of the bacillary-antisera, in contradistinction to the bacillary-saline specimens, will no longer appear uniformly granular, but that "wall patterns" composed of masses of agglutinated organisms with clear interspaces will have formed. Make drawings to illustrate appearances at the beginning and the ending of the hour, (*a*) as seen with the low power; (*b*) as seen with the middle power. The adjustment of the latter must be carried out with care, so as not to force the mixture through the vaselin wall. Note with the middle power that the previously actively motile organisms have become immobilized by the antisera, while in the saline controls the motility remains as in the beginning. Draw and append explanatory legends.

EXPERIMENT C.—*Macroscopic Test-tube Method.*—Charge three agglutination tubes with 0.5 c.c. each of the three bacillary emulsions and label accordingly (1) T; (2) PTa; (3) PTb; add to each bacillary emulsion 0.5 c.c. of the corresponding antiserum of moderate titer; put up corresponding saline control tubes and crossed mixtures—*e. g.*, 0.5 c.c. of typhoid emulsion plus 0.5 c.c. of anti-paratyphoid serum; close all tubes with little cotton stoppers and set them aside at room temperature until the following day. Then note that a granular sediment covered by a clear supernatant fluid will have formed in the homologous mixtures, while the saline controls remain turbid and the cross mixtures show only an inconclusive reaction. Draw the appearances and append explanatory legends.

Answer the Following Questions in Writing.—What is meant by the terms (*a*) antigen, (*b*) antibody? Give classification of the different antibodies and the corresponding antigens, arranged in tabulary form. How would you define an agglutinin? What term is applied to the corresponding antigen? Who discovered the agglutinins? In what manner did Widal's name become connected with the history of the agglutinins? Is the agglutination reaction used for diagnostic purposes outside of the study of typhoid fever? Why is it necessary to dilute the patient's serum in doing the Widal test? How early in the disease does it appear? How long does it persist after recovery? Does a positive Widal invariably indicate that the patient is suffering at the time or has been suffering in the past from typhoid fever? Is it essential that the bacilli used in the test be alive? How may they be preserved for agglutination purposes? Will the serum of a paratyphoid patient agglutinate typhoid bacilli? How would you distinguish between a typhoid and a paratyphoid case serologically? What is meant by spontaneous agglutination? How would you proceed to rule out spontaneous agglutination in doing the agglutinin test in a suspected case of paratyphoid fever?

Reading.—

Lesson 11.—*Apparatus and Reagents Required.*—Same as for Lesson 10. In addition supply two specimens of fecal material—particles the size of a pea, emulsified in about 10 c.c. of saline, one of which is to contain typhoid bacilli in addition to the usual flora; the other should contain no typhoid organisms. Do not indicate on the label which is which, but mark x and y. Supply every two students with one Drigalski-Conradi plate, one Endo-plate, a Bril-

liant green plate and a Russell plate; use large plates and fill them to a depth of at least 3 mm. Have also available a colon culture, besides the typhoid culture.

Experiment A.—Collect about 10 drops of blood from your working partner's ear, using a small agglutination tube for the purpose, dipping up the drops as they are squeezed out. Allow the blood to clot; loosen the clot from the walls of the tube with your nichrome wire or a sealed capillary pipette; centrifugalize; pipette off the supernatant serum and dilute with saline 1 to 25, using an agglutination tube; label 1 to 25. With this diluted serum do the microscopic slide agglutination test and the macroscopic test-tube test, as described in the previous lesson. Read the result of the first at the end of one hour and that of the second the next day.

Answer the Following Questions in Writing.—What result did you obtain in examining your partner's blood? If positive did you ascertain whether he has had typhoid fever in the past and when? If he gives no history of typhoid, did you ascertain whether he has been vaccinated against typhoid and could that give rise to a positive reaction? Does a Widal reaction occur in typhoid carriers? If you found a positive Widal in a female, a number of years after an attack of typhoid, and if you also obtained a history of gall-stone attacks in the individual, what would your course of procedure be and why?

Experiment B.—With a sterile sealed capillary pipette divide each one of your culture plates into four quadrants and mark these with a blue pencil on the bottom of the plate. Place a minute drop of the typhoid culture (in bouillon) on quadrant I and spread it out over the entire quadrant with your nichrome wire or a bent and sealed capillary pipette; smear quadrant II with a droplet of the colon culture, quadrant III with the fecal emulsion *x* and quadrant IV with the fecal emulsion y. Cover the plates, turn them over and incubate until the next day.

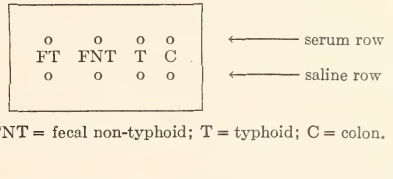
Answer the Following Questions in Writing.—What is the composition of (*a*) the Conradi-Drigalski medium; (*b*) of the Endo medium; (*c*) the brilliant green medium; (*d*) the Russel medium? What role does the anilin dye play in *a*, *b* and *c* respectively? What is Andrade's indicator? what does it indicate?

Reading.—

Lesson 12.—Continuation of Lesson 11.—*Reagents Required.*—The same as in the preceding lesson, plus four tubes of glucose bouillon

and of litmus milk for each man. Examine the plates which you incubated yesterday. Note and draw with colored pencils the appearances of the typhoid and colon colonies in quadrants I and II. Carefully examine the colonies in the remaining quadrants and by comparison with those of I and II ascertain which fecal specimen contained typhoid bacilli and which was free of them. Draw these appearances also. Prove that the colonies which you suspect of being typhoid are in reality so (*a*) by the macroscopic slide agglutination method, (*b*) by culture in glucose bouillon and in litmus milk. Do controls with the colonies obtained from quadrants I and II, as well as with those from the fecal non-typhoid quadrant and the non-typhoid colonies of the fecal typhoid quadrant.

In doing the macroscopic slide agglutination test mount a series of good-sized drops of the high titer antityphoid serum (1 to 100) in one row (top) and a second row of saline drops below this, so that a saline drop corresponds to a serum drop. Fish a colony that you wish to examine with the nichrome loop, emulsify it in the saline drop and transfer a bit of the emulsion to the serum drop; or emulsify a colony of the same kind in the latter; label your specimens with the glass pencil.



FT = fecal typhoid; FNT = fecal non-typhoid; T = typhoid; C = colon.

The drops should not be too small, otherwise they will dry up before a result can be obtained. Observe the drops in the top row with the naked eye on a black background—stage of the microscope—or with the low power and note the development of clumps of agglutinated bacilli. Illustrate the final appearance of all the drops and append appropriate legends.

Answer the Following Questions in Writing.—What is meant by a typhoid carrier? Do typhoid bacilli occur in the fecal contents of typhoid patients as a rule? If so, how long into convalescence do they persist? Do they usually persist? What is meant by a chronic carrier? What is meant by an active, and what by a passive carrier? What relationship exists between gall-bladder infection and the carrier state? Are female carriers more common than male carriers,

or *vice versa?* Which are more common, fecal or urinary carriers? Is the elimination of organisms in fecal carriers continuous or intermittent?

What is the reason of the difference in the appearance in your plates of the typhoid as compared with the colon colonies: (*a*) on the Drigalski plate, (*b*) on the Endo plate, (*c*) on the brilliant green plate, (*d*) on the Russell plate?

What is the difference in behavior of the typhoid as compared with the colon bacillus (*a*) in glucose bouillon, (*b*) in litmus milk? What other bacilli occurring in the feces are alkali producers? How would you distinguish between them and the typhoid bacillus? What other bacilli besides the colon bacillus, occurring in the feces are acid producers?

Reading.—

Lesson 13.—*Apparatus and Reagents Required.*—Furnish every student with one agglutination test-tube rack holding a dozen tubes; four test-tubes of ordinary size; one clean tumbler; one 1 c.c. pipette, graduated in $\frac{1}{100}$s; a dozen capillary pipettes with rubber nipples; a wax pencil.

Supply every six men with 250 c.c. of sterile 0.9 per cent. saline; 50 c.c. of a 2.5 per cent. washed sheep corpuscle emulsion; 10 c.c. of diluted fresh complement (1 to 10), or using acetated complement (one part of guinea-pig serum, treated with one and a half part of a 10 per cent. solution of c. p. sodium acetate in 0.9 per cent. saline), dilute 2.5 c.c. of the acetated complement with 7.5 c.c. of saline; 10 c.c. diluted amboceptor—strength to equal three times that of the titer; 10 c.c. of diluted cholesterinized antigen—strength to equal two-thirds that of the titer; 2.5 c.c. of an actively fixing syphilitic serum and a like quantity of a non-fixing serum; 10 c.c. and 1 c.c. pipettes graduated in $\frac{1}{10}$ and $\frac{1}{100}$ c.c. respectively to accompany the reagents.

Have available also one water-bath, at 56° C., and one water-bath at 37° to 40° C., provided with racks for tubes; an electric or high-speed water-power centrifuge, preferably with many arms (laboratory technician should supervise work at centrifuge).

Directions to the Student.—Provide yourself from the stock reagents set out for every six men with 40 c.c. of saline—put this in your tumbler; 8 c.c. of sheep corpuscle emulsion, put this in a test-tube; 1.5 c.c. each of diluted complement, amboceptor and antigen; and 0.4 c.c. each of the syphilitic (I) and the non-syphilitic

(II) serum; put these into your small test-tubes; now proceed with the following experiment:

EXPERIMENT A.—Mark four agglutination tubes serially 1, 2, 3 and 4. Charge the tubes as follows: (1) with 0.2 c.c. corpuscle emulsion and 0.4 c.c. saline; (2) with 0.2 c.c. corpuscle emulsion, 0.2 c.c. diluted complement and 0.2 c.c. saline; (3) with 0.2 c.c. corpuscle emulsion, 0.2 c.c. diluted amboceptor and 0.2 c.c. saline; (4) with 0.2 c.c. corpuscle emulsion, 0.2 c.c. diluted amboceptor and 0.2 c.c. diluted complement.[1] Incubate in the water-bath at 37° to 40° C., or in the absence of such, in your upper vest pocket. Shake every few minutes. Note the result at the end of fifteen minutes. The occurrence of hemolysis is indicated by laking of the blood cells. Centrifugalize your tubes and note the appearance of the fluid, whether colorless or blood-tinged, and the appearance or non-appearance of a sediment of non-hemolized red cells, at the bottom. Arrange your findings in tabulary form, stating under the heading "results" whether hemolysis has or has not occurred; whether it is complete or not, *e. g.*:

Components.	Period of incubation.	Result.
Tube (1) corpuscles + saline.	Fifteen minutes.	No hemolysis.

EXPERIMENT B.—Repeat experiment A–4, (*a*) using complement that has been previously kept at a temperature of 56° C. for fifteen minutes and (*b*) using amboceptor that has been treated in the same manner. Note the results and compare with those obtained sub A–4.

EXPERIMENT C.—*First Step.*—Place your 0.4 c.c. portions of syphilitic and non-syphilitic serum in agglutination tubes, marked I and II respectively, and keep in the 56° C. water-bath for fifteen minutes.

Second Step.—Add 2 c.c. of the sheep corpuscle emulsion to each tube; shake well and incubate at 37° to 40° C. for ten minutes.

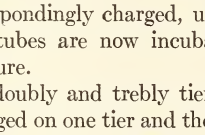
Third Step.—Centrifugalize until the supernatant fluid is perfectly clear; pipette this off by the aid of a clean capillary pipette, marking the tubes I and II as before.

Fourth Step.—Mark four agglutination tubes IA, IC, IIA and IIC respectively. Charge IA with 0.2 c.c. of serum I, treated as just directed; add 0.2 c.c. of the diluted antigen and 0.2 c.c. of

[1] If suitable graduated pipettes are not available, use capillary pipettes, allowing 3 drops for 0.2 c.c.; wash out the pipette with a few changes of saline before taking up another reagent.

the diluted complement. Charge IIA in a corresponding manner, using serum II in the place of serum I. Tube IC is the control to IA and receives 0.2 c.c. of the serum, 0.2 c.c. of the diluted complement and 0.2 c.c. of saline instead of the antigen. Tube IIC is correspondingly charged, using serum II instead of serum I. The four tubes are now incubated for fifteen minutes at body temperature.

If doubly and trebly tiered racks are available the A tubes are arranged on one tier and the C tubes immediately behind the others; they may then be charged *in situ*, as shown in the diagram:



At the expiration of the fifteen minutes each tube—both of the A row and the C row—now receive 0.2 c.c. of the diluted amboceptor and 0.2 c.c. of the corpuscle emulsion. The tubes are well shaken and returned to the water-bath, where they remain for fifteen minutes. At the end of that time it will be noted that the contents of the C tubes are entirely hemolyzed and perfectly clear. Centrifugalize the A tubes and compare the result in tube I with that obtained in tube II. Note the color of the supernatant fluid and the presence or absence of non-hemolyzed red cells at the bottom. Arrange your findings in tabulary form; under the heading "Results," state whether or not hemolysis has occurred, whether there is none whatever, or whether it is partial or complete, *e. g.:*

Serum 1 to 6.	Complement 1 to 10.	Antigen 1 to 10.	Incubate at 37° C. for 15 minutes.	Amboceptor 1 to 1000.	Corpuscle emulsion 2.5 per cent.	Incubate at 37° C. for 15 minutes.	Result
0.2 c.c. A	0.2 c.c.	0.2 c.c.		0.2 c.c.	0.2 c.c.		No hemolysis.
0.2 c.c. C	0.2 c.c.	0.2 c.c.		0.2 c.c.	0.2 c.c.		Complete "

Answer the Following Questions in Writing.—What is the source of the complement usually employed in the complement-fixation tests? How is the reagent prepared, ready for use? What is acetated complement and how is it prepared? How is the sheep corpuscle emulsion prepared? What is the source of the antisheep

amboceptor? How is it obtained and how is it kept? What is meant by the titer of (*a*) the amboceptor, (*b*) the complement? What is the effect of heating at 56° C. upon complement? How is the amboceptor affected under like conditions? Illustrate diagrammatically the manner in which hemolysis is produced and the role played by complement and amboceptor. In doing the Wassermann test, why do you first heat the serum at 56° C.? Why do you subsequently treat it with a sheep-cell emulsion? What is meant by an antisheep hemolytic system? On what principle is the Wassermann test based? What role is played in the test, respectively, by the patient's serum? the antigen? the complement, the amboceptor and the corpuscles? What is the object of the incubation at 37° C. after bringing the patient's serum, the antigen and the complement together? Why do you finally add the sheep corpuscles and the amboceptor? Why does hemolysis not occur in the end if the patient's serum was syphilitic? and why does it take place if the serum was non-syphilitic? What does the term "complement fixation" denote in connection with the test in question? What is the active principle of the antigen used in the Wassermann test? What is the source of the antigen in question? How is it prepared? What is meant by cholesterinized antigen? What is the idea underlying the preparation of Noguchi's antigen? What is meant by the titer of the antigen?

Reading.—

Lesson 14.—*Apparatus and Reagents Required.*—For every six men furnish of alcoholic beef-heart extract 50 c.c.; of cholesterinized beef-heart extract the same quantity; 250 c.c. of saline; 50 c.c. of a 2.5 per cent. emulsion of washed sheep corpuscles; 30 c.c. of diluted, fresh complement (1 to 10), or using acetated complement dilute 2.5 c.c. with 7.5 c.c. of saline; 30 c.c. of antisheep amboceptor, 1 to 1000.

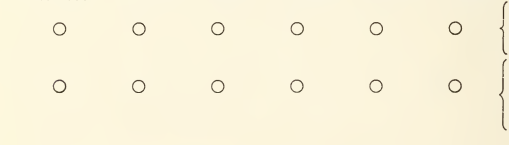
Every student to be supplied with two dozen agglutination tubes; an agglutination test-tube rack; capillary pipettes; rubber nipples; 1 c.c. pipette graduated in hundredths; a wax pencil, as in Lesson 13.

A water-bath kept at 56° C., one at 37° to 40° C., and an electric centrifuge should also be available. As in the preceding lesson the technician should supervise the work at the centrifuge.

Directions to the Student.—Provide yourself from the stock reagents set out for every six men, with 40 c.c. of saline; 8 c.c. of

sheep corpuscle emulsion; 5 c.c. of diluted complement; 5 c.c. of antisheep amboceptor and 6 c.c. each of the plain beef-heart extract and the cholesterinized extract; mark all your reagents with the wax pencil.

EXPERIMENT A.—*Determine the Titer of the Amboceptor.*—Of the 1 to 1000 dilution furnished, prepare subdilutions as follows: Mark six agglutination tubes 1000, 2000, 3000, 4000, 5000 and 6000 respectively; place them in your rack (first row) and charge each one with 0.2 c.c. of the 1 to 1000; to the second tube add 0.2 c.c. saline; to the third 0.4 c.c., to the fourth 0.6 c.c., to the fifth 0.8 c.c. and to the sixth 1.0 c.c. Set up a second row of five tubes, marked from 2000 to 6000 and place these in the second row behind those of the first; move your 1 to 1000 tube, as it is, to the second row to its proper place. Then transfer 0.2 c.c. of each dilution in the front row, to the corresponding tube in the back row (see diagram).



To each tube of the second row add 0.2 c.c. of the diluted complement and 0.2 c.c. of the corpuscle emulsion; shake well and incubate for fifteen minutes in the water-bath at 37° C., shaking from time to time. At the expiration of the fifteen minutes examine your tubes and note the extent to which hemolysis has taken place. The highest dilution which has cleared absolutely indicates the titer of the amboceptor. In the actual Wassermann test or in titrating the antigen (see below) three times the titer strength is used, *e. g.*, if the titer was 1 to 5000 we should use a dilution that is three times as strong; in the present instance this would be $\frac{5000}{3} = 1666 = 1$ to 1666. Every remaining cubic centimeter of the 1 to 1000 dilution would hence be further diluted by adding 0.66 c.c. of saline. Do this and label the resultant amboceptor dilution correspondingly.

EXPERIMENT B.—*Determine the Titer of Both Antigens* as follows: Set up a series of six agglutination tubes and mark them 3 to 10, 2.5 to 10, 2 to 10, 1.5 to 10, 1 to 10, 0.5 to 10. Charge the tubes respectively with 3.5, 3.75, 4.0, 4.25, 4.5, 4.75 c.c. of saline, and add of the antigen to be examined 1.5 c.c. to the first, 1.25 c.c. to the second, 1.0 c.c. to the third, 0.75 c.c. to the fourth, 0.5 c.c. to the fifth

and 0.25 c.c. to the sixth, using the 1 c.c. pipette, graduated in hundredths, washing it out well with saline after use. Mix the constituents well. Mark a second set of tubes in the same manner as the first set and arrange them in the rack in the second row, so as to correspond to those in the first. Transfer from the mixtures in the first row 0.2 c.c. to the corresponding tubes in the second row, starting with the highest dilution, *i. e.*, the 0.25 to 4.75 which represents a dilution of 0.5 to 10. Add to each tube of the second row 0.2 c.c. of the diluted complement and 0.2 c.c. of saline; mix well and incubate for ten minutes at 37° C.; then add 0.2 c.c. of amboceptor, using three times the titer strength and 0.2 c.c. of the sheep corpuscle emulsion; mix well and reincubate for fifteen minutes; then centrifugalize and note the results, viz., whether complete hemolysis has taken place in any one of the tubes. The highest concentration of the antigen dilution in which complete hemolysis was obtained represents the titer. In the actual Wassermann test the antigen is used in a concentration corresponding to two-thirds the titer dose. Write down your results in tabulary form; indicate the titer and the concentration to be used in the actual Wassermann Test.

After having determined the titer of the plain beef-heart extract, ascertain that of the cholesterinized antigen, proceeding in the same manner. Wash out your tubes, first with water, then with saline.

Answer the Following Questions in Writing.—The titer of an antigen was 1.5 in 10; how much saline and undiluted antigen respectively would you use to make up a total bulk of 5 c.c. for actual work? You are to examine 10 sera for the Wassermann reaction and to use 0.4 c.c. of every diluted reagent, as standard volume, *employing two antigens;* the titer of your amboceptor is 1 to 3000; how many cubic centimeters of how strong a dilution are you going to make up to cover your needs? Give your calculation in detail. How many cubic centimeters of native complement will you need, and how many cubic centimeters of corpuscle emulsion? How is acetated complement prepared? Starting with acetated complement, how many cubic centimeters would you need and how much saline would you add to examine the ten sera in question? How much hashed beef-heart would you use to make up 200 c.c. of plain heart extract? How much 1 per cent. cholesterin solution, and how much of the plain alcoholic extract would you use to make up 100 c.c. of the mixture? How would you ascertain whether a rabbit that you have

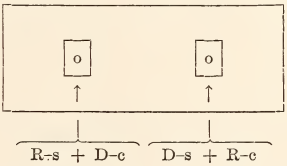
been immunizing against sheep cells is ready to be killed? How would you store its serum? Of what strength should your stock solution be? How would you keep the latter? Why does Noguchi recommend the use of an antihuman hemolytic system? How do others overcome the same objection attaching to the use of the anti-sheep system? What does the writer mean by an instantaneous Wassermann reaction? What would you do if a patient's serum only showed partial complement fixation after fifteen minutes' incubation? How would you express your findings if complement-fixation resulted only after forty-five minutes' incubation? How would you express your findings if you obtained an instantaneous reaction?

Reading.

Lesson 15.—TRANSFUSION TEST.—*Apparatus and Reagents Required.*—Sterilized gauze sponges in pairs, wrapped in muslin; Hagedorn needles; individual bottles of alcohol (2 oz.); agglutination tubes, such as were used for collecting blood for the Widal reaction—two for each student; 0.9 per cent. saline, containing 1.5 per cent. sodium citrate, 10 c.c. for every six men; 0.9 per cent. saline, 150 c.c. for every six men; 2 c.c. of an antihuman serum (1 to 1000); two capillary pipettes with rubber nipple for every man; clean slides and cover-glasses; vaselin in tubes, one for every two men; wax pencils, nichrome wire; an incubator at 37° to 40° C.; electric centrifuge.

Directions to the Student.—Supply yourself from the stock reagents with about 1 c.c. of the citrate solution; place this in one of your agglutination tubes; take about 25 c.c. of saline and keep this in a little whiskey glass or beaker. Mark your reagents with the wax pencil. Puncture your working partner's ear with the Hagedorn needle, after cleaning it with alcohol and drying it, in the usual manner. Collect about eight or ten drops of blood in a dry agglutination tube; after clotting has occurred, loosen the clot with your nichrome wire and centrifugalize. Also collect about five or six drops of the same person's blood in the little tube, containing the citrate solution. Regard yourself as the recipient of your working partner's blood and his serum and corpuscles accordingly as the donor's. Your working partner will similarily regard himself as the recipient of your serum and corpuscles, and you accordingly as the donor. Mark serum and corresponding corpuscles with the name of the individual from whom they were obtained.

Prepare a couple of vaselin rings on the slide, side by side, and mark them R–s (recipient's serum) and D–c (donor's corpuscles) and D–s (donor's serum) and R–c (recipient's corpuscles). By the aid of one of your capillary pipettes charge the little vaselin chambers as indicated, mixing two large drops of the respective serum that is called for, with one small drop of the corresponding corpuscle emulsion. Wash out the pipette after the use of every reagent, with a few changes of saline from your little beaker. Adjust a cover-glass over each ring, as in your Widal work, pressing it into position, until it touches the drop and is everywhere in contact with the vaselin ring. Examine the specimen with the middle power of your microscope, and note that the red cells all show their greenish-yellow color and that there are no blood shadows (colorless rings) or at most a few isolated ones. Incubate the slide for thirty minutes and reëxamine. If either serum contained hemolysins the corpuscles of the corresponding mixture will either have disappeared entirely or blood shadows will be present in large numbers—extensive hemolysis may be recognized with the naked eye by the occurrence of extensive clearing of the specimen. If either serum contained agglutinins the corpuscles of the specimen will be gathered in clumps which are usually visible with the naked eye. If neither hemolysins nor agglutinins are demonstrable the blood of the donor may be regarded as entirely suitable for transfusion purposes, providing of course the donor is not syphilitic nor suffering from bacteriemia. Slight agglutination may be disregarded. If extensive agglutination occurs it is advisable to seek another donor. If hemolysis occurs the donor should of course be rejected.



If in the above experiment the sera examined were free from any hemolytic action prepare a vaselin ring on another slide and mount a mixture of one drop of your corpuscle emulsion with one drop of the anti-human (rabbit) serum supplied to your group; adjust a cover-glass and incubate for a few minutes; examine with the low power at frequent intervals, and as soon as you see any evidence of hemolysis look for blood shadows with your middle power.

Answer the Following Questions in Writing.—What technical term is applied to hemolysins which will hemolyze the red cells of an animal of the same species? Why would the presence of such hemolysins in the serum of either the recipient or the donor, in connection with a contemplated transfusion, be objectionable? Why would the presence of agglutinins under like conditions be objectionable? What is meant by hemoglobinemia? What is meant by autohemolysins? What relation exists between the occurrence of paroxysmal hemoglobinuria and the activity of autohemolysins?

Reading.—

Lesson 16.—Blood-sugar Estimation.—*Apparatus and Reagents Required.*—Provide every two students (working partners) with the following: 25 to 30 c.c. of a saturated aqueous solution of picric acid; a similar quantity of a 10 per cent. aqueous solution of sodium carbonate; a tumblerful of water; 1 or 2 c.c. of a 2.0 per cent. solution of sodium fluoride; a 1 c.c. pipette, graduated in tenths; a capillary pipette with rubber nipple; a 10 c.c. pipette graduated in tenths; a boiling test-tube of similar capacity—of as wide a diameter as possible; a Sahli hemoglobinometer calibrated tube; a standard color tube both of the A and the B type, such as are furnished with the Kuttner microcolorimeters; one colorimeter stand of the latter type. Bunsen burners and centrifuge.

Supply every six students with a few cubic centimeters of ordinary oxalated human blood, which may be collected in vacuum tubes containing a little powdered sodium oxalate, or by the open method, allowing blood to run through a needle from a vein directly into a tube containing a little oxalate or citrate; in either case the mixture should be well shaken, immediately after the blood has been drawn; mark this (I). If possible secure a specimen of diabetic blood also (II).

Directions to the Student.—Of specimen I place 0.2 c.c. in the 10 c.c. graduated test-tube, rinse the pipette two or three times with water, adding the rinsings to the blood; dilute with water to the 1 c.c. mark and add picric acid solution up to the 2.5 c.c. mark. Shake well and centrifugalize. Pipette off the clear supernatant fluid and place 1 c.c. in the boiling test-tube furnished you. Be careful, picric acid is poisonous. Boil down to two or three drops, shaking all the time, so that the tube does not crack. Add 0.5 c.c. of the 10 per cent. solution of sodium carbonate, having washed out your 1 c.c. pipette, after using it to measure off the

picric acid mixture. Boil again until the last few drops undergo crystallization on removal from the flame; note the change in color to brownish red. Dissolve the residue with a few drops of water, by the aid of a little heat, and transfer the solution to the calibrated tube of the colorimeter; rinse with a few drops of water at a time and add the rinsings to the solution; dilute to the 50 mark and compare the color with the standards A and B. If it is darker than A, but lighter than B, the former is used as standard; the mixture is then diluted with water, as in your hemoglobin estimation, until the colors match. To ascertain this point it is well to compare them, while the tubes stand in the little colorimeter, the slide of the latter being lowered down to the blending prism. Read off the figure reached; divide by 1000; the resultant number indicates the percentage of sugar in the specimen examined. If the darker tube B is used the result must be multiplied by 2.

Repeat the examination with specimen II, which was obtained from a diabetic patient. In this case start your work, using 0.1 instead of 0.2 c.c. of blood and multiply all your figures in the end by 2. After completing your work, carefully wash out all your tubes and pipettes—using plain water—and if necessary a little alcohol. In noting your results indicate clearly how much blood you used to start with, what your readings were and how you arrived at your final figures.

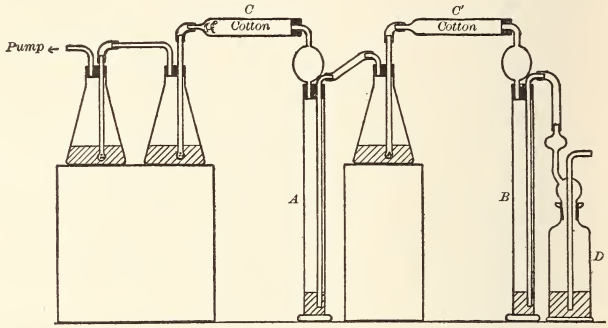
Repeat the examination with your working partner's blood, collecting the necessary amount from a free ear-puncture and allowing the blood to flow into a little tube containing a drop of a 2 per cent. solution of sodium fluoride.

Answer the Following Questions in Writing: What is the normal sugar content of the blood? What is meant by hyperglycemia? Under what conditions does this occur? Contrast the findings obtained in a case of alimentary hyperglycemia following the administration of 100 grams of dextrose, with the result that you would be most liable to obtain in a diabetic under like conditions.

Reading.—

Lesson 17.—Blood Urea Estimation.—*Apparatus and Reagents Required.*—On the day of the experiment furnish every two students with two cylinders of approximately 150 c.c. capacity each; two Erlenmeyer flasks of 200 c.c. capacity; two Folin absorption tubes; two calcium chloride tubes, filled with cotton; and the necessary glass tubing, rubber tubing and rubber stoppers to set up the

arrangement shown in the accompanying illustration. Furnish also a Mohr's burette with stand.



Supply every six men with one absorption bottle (see *D* in illustration), containing 30 per cent. sulphuric acid; so also 500 c.c. $\frac{N}{5\,0}$ sulphuric acid, 500 c.c. $\frac{N}{5\,0}$ sodium hydroxide solution; 500 c.c. of distilled water; 50 c.c. of alcohol; 50 c.c. of kerosene oil; a dozen 1 gram sodium chloride tablets; 4 grams of sodium carbonate and 1 ounce of a 1 per cent. aqueous solution of alizarin. One suction pump should be available for every six men. On the day preceding the actual experiment supply every two students with two separate portions of 5 c.c. of oxalated blood, obtained from a case of advanced nephritis. The student should place each portion in a test-tube labelled A and B respectively. Tube A receives 25 mg. of urease-soy bean extract (Hynson, Westcott & Dunning tablets), which is preferably placed in the tube, pulverized and suspended in 2 or 3 c.c. of water, before the blood is added. Both specimens are then layered with 0.5 to 1 c.c. of toluene, well shaken, tightly corked and kept overnight at room temperature.

Directions to the Student.—Transfer the contents of tube A to cylinder A by the aid of a few cubic centimeters of water, not more than five; add an equal quantity of alcohol, 2 grams of sodium chloride—two 1-gram tablets which should first be broken into small pieces, and a layer of kerosene oil, about 2 c.c. The contents of tube B are placed in cylinder B and treated like A. 25 c.c. of $\frac{N}{5\,0}$ sulphuric acid are placed in each one of the two Erlenmeyer flasks, with which you have been provided, and diluted with a similar

quantity of distilled water. 0.5 to 1 gram—a good knifepointful —of sodium carbonate is finally added to each cylinder, when cylinders and flasks are tightly closed with their respective stoppers, united with each other and connected with a suction pump, as shown in the illustration (eliminating the flask at the extreme left). All three sets of apparatus from your group are connected with the same pump, the absorption bottle D being placed at the extreme right end. An air current is now passed through the entire system for a period of one hour. At the end of this time the current is stopped, the groups of apparatus and their individual components are disconnected, and the contents of flasks A and B titrated with $\frac{N}{50}$ alkali to the violet point, using four drops of alizarin as indicator. Deduct the amount of ammonia evolved in B from A. You will then find how many cubic centimeters of the 25 of $\frac{N}{50}$ acid were neutralized by the ammonia resulting from the decomposition of the urea contained in the quantity of blood with which you started. From this calculate the amount of urea per 1000 c.c. of blood, bearing in mind that 0.6 mgm. of urea will give rise to a quantity of ammonia corresponding to 1 c.c. of $\frac{N}{50}$ acid. When noting your results show exactly, step by step, burette readings included, how you arrived at your final figure.

Answer the Following Questions in Writing.—How much urea is normally found in the blood, per liter? How much would you expect to find in the case of a patient whose kidneys are more or less diseased? What prognostic conclusion would you draw from the quantity found? Write the equation which expresses the transformation of urea into ammonium carbonate, as the result of the action of the urease. Why do you add sodium carbonate to the mixture, in each cylinder? Why do you deduct value B from value A? What do you mean by the non-protein nitrogen of the blood? Name the three most important substances belonging to this order? Retention of which one of the three is most apt to occur first, in the course of a developing nephritis? Which next and which last? What are the fluctuations in quantity of the non-protein nitrogen in the absence of renal disease? What percentage of the total is represented by the three most important components, individually? What values would you expect in a case of chronic nephritis associated with hypertension? Under what conditions would you expect a material increase in the amount of the so-called free ammonia?

Reading.—

Lesson 18.—The Microscopic Examination of Transudates and Exudates.—*Material Required.*—Smears on slides of the cellular components (*a*) of a typical transudate; (*b*) of a transudate obtained from a case of peritoneal carcinomatosis; (*c*) of an exudate obtained from a case of tubercular pleurisy or peritonitis; (*d*) of an exudate obtained from a pyogenic peritonitis; (*e*) of pus from a case of empyema; (*f*) of pus from a case of gonorrheal urethritis. The smears *a* to *d* should be fixed by heat and stained with a 1 per cent. aqueous solution of methylene-blue or with carbol thionin; *e* to *f* should be stained with Jenner's stain.

Directions to the Student.—In specimen (*a*) note the comparative scarcity of the cells and the types—endothelial cells and small mononuclear leukocytes; draw these types and differentiate 50 cells; give percentage values. In specimen (*b*) note the types—endothelial cells, tumor cells, showing evidence of atypical mitosis, leukocytes; note the large number of cells in general and of cells presenting an epithelial habitus; differentiate 100 cells and give percentage values. Draw the different types. In specimen (*c*) note the number of cells and the types—leukocytes and endothelial cells; differentiate 100 cells and give percentage values. Sketch a representative field. In specimen (*d*) note the number of cells; differentiate 100 cells and give percentage values; note the number and types of bacteria and the occurrence of phagocytosis on the part of many of the cells. Sketch a representative field. In specimen (*e*) note the same factors as in (*d*); observe also the absence or scarcity of eosinophiles; differentiate 100 cells and give percentage values. Sketch a representative field. Study specimen (*f*) in the same manner as (*e*); note the presence of eosinophiles and epithelial cells; study the morphological appearance of the gonococci and their occurrence within the polymorphonuclear neutrophiles; differentiate 100 cells and give percentage values. Sketch a representative field.

Answer the Following Questions in Writing.—What is the essential difference between a transudate and an exudate? What is meant by the term "cytological study" of the various effusions? Generally speaking, how would you distinguish on cytological grounds, between an exudate of tubercular origin and one due to pyogenic organisms? Does a lymphocytosis invariably indicate tuberculosis? What influence does chronicity of the pathological process which gives rise to the formation of an effusion, have upon the character of the cytological findings? What organisms are most apt to be

encountered in exudates? In a suspected case of tubercular effusion, what examination, aside from a microscopic one of the cellular elements, would suggest itself to you as advisable? How would you proceed? What would you expect to find in a positive case? What is meant by a chylous exudate? Under what pathological conditions may such be found? To what is the chylous appearance due?

Reading.—

Lesson 19.—*Material, Apparatus and Reagents Required.*—Furnish a tubercular specimen of sputum, in Board of Health vials, to every six men; so, also, approximately, 10 c.c. of antiformin and an equal amount of a mixture composed of 1 part of chloroform and 9 parts of alcohol. Supply every two students with 1 or 2 oz. of a 1 per cent. aqueous solution of methylene-blue; of Jenner's stain; of carbol fuchsin and Gabbett's methylene-blue-sulphuric acid mixture. Staining trays and wash bottles, filled with ordinary tap water, should also be available, besides Bunsen burners, slides, nichrome wires and individual paper napkins. Provide basins containing 10 per cent. lysol and an Arnold sterilizer for disinfecting purposes.

Directions to the Student.—Each student should prepare two fairly thin smears from the most purulent portion of the sputum and one or two thicker smears and mark them (*a*), (*b*) and (*c*) respectively. The specimens should either be allowed to dry in the air or by "beating" the flame of the Bunsen burner—touching the hand after every few beatings, so as to prevent overheating. After being dried in this manner stain specimen (*a*) for a minute or so with the aqueous methylene-blue solution; lay the slides upon the carrying rods of the tray and flood them with the stain; wash off with water, cleanse the reverse side with a paper napkin and dry the smear by beating the Bunsen flame. Stain (*b*) with Jenner's stain in the same manner as you would stain a blood smear; do not dry by heat after staining. To stain specimen (*c*) raise up the carrying frame from the staining tray and set it obliquely across the latter; lay your slide across the rods, flood it with carbol fuchsin solution, bring this to steaming by heating with the Bunsen flame; keep the solution at the steaming point for a couple of minutes, adding more stain, if need be, to keep the smear well covered; wash off with water; flood the slide with the acid-methylene-blue mixture; leave this for three or four minutes; wash off with water; cleanse the reverse side of

the slide with a paper napkin; dry the smear by beating the Bunsen flame.

To the remainder of the sputum in the vial, add approximately one-fourth the quantity of antiformin and allow the mixture to stand at room temperature, well stoppered, until the next day, or in the incubator at 37° to 40° C. for one hour, shaking from time to time. After the mixture has become homogeneous, add for every 10 c.c. approximately 1.5 c.c. of the solution composed of 1 part of chloroform and 9 parts of alcohol; shake. Pour the mixture into centrifugalizing tubes—one for every two men of your group of six; centrifugalize for fifteen minutes; pour the supernatant fluid into the lysol basin, make smears from the residue; fix by heat and stain with carbol fuchsin and acid methylene-blue, as directed above; mark these slides (*d*). Place a drop of immersion oil upon each one of your smears and examine with the oil-immersion lens.

Note in (*a*) that the cellular elements are for the most part polymorphonuclear or polynuclear leukocytes, but that small mononuclear leukocytes also are present; search for round or oval "alveolar" epithelial cells and for irregular squamous epithelial cells derived from the pharyngeal district; note the large number of bacteria and the predominance of cocci, some in diplo, others in chain form and still others occurring singly or in groups; cocci predominate over bacilli. Sketch a representative field. In the Jenner specimen (*b*) note that among the leukocytes there is an occasional eosinophile; look also for the presence of red cells. In specimen (*c*) you will observe that the various cellular elements and bacteria which you noticed in (*a*) and (*b*) are not so sharply brought out. The blue here merely serves as a background stain to facilitate the finding of any tubercle bacilli that may be present. The latter are colored red. Note their size, their frequent arrangement in groups of two or three, or in clumps of larger numbers; look for "beaded" specimens and organisms united so as to resemble in form the letter S. Make a composite sketch of your findings. In specimen (*d*) the cellular elements and the other organisms seen in (*a*), (*b*) and (*c*) will be missed; they have been destroyed by the antiformin; the tubercle bacilli will be found to be more numerous, as a result of the concentration of the sputum. At the end of the lesson place all sputum containers and soiled material in the steam sterilizer and run this for thirty minutes.

Answer the Following Questions in Writing.—What elements give rise to the opaque appearance of the sputum? What is the probable

origin of the greenish color of the sputum? What epithelial elements are likely to be found in sputum? What cell is the common pus corpuscle? What kinds of bacteria, besides the tubercle bacillus, would you expect to find in sputa coming from a tuberculous patient? In what respect does the tubercle bacillus differ from most of the associated organisms found in sputa in reference to its behavior toward the usual bacterial stains, when used in aqueous or alcoholic solution? What is meant by a mordant? Name the two mordants which are most commonly employed in staining the tubercle bacillus? What role does the sulphuric acid in Gabbett's stain play in the demonstration of the tubercle bacillus? Explain the term acid-fast. Name other bacilli that are acid-fast and where are they found? Of what significance is the presence of elastic tissue in sputum?

Reading.—

Lesson 20.—*Material, Apparatus and Reagents Required.*—For every six students supply the following: A specimen of sputum from a case of pneumococcus pneumonia; a couple of old forceps; a large basin with 10 per cent. lysol solution; half a dozen tubes of meat infusion broth, 0.3 to 0.5 per cent. acid to phenolphthalein, 5 c.c. to the tube; 5 c.c. of a sterile 20 per cent. solution of dextrose; 5 c.c. of sterile, defibrinated human or rabbit blood; wash bottles with water.

Furnish every two students with a couple of sterile tumblers with sterile saline—closed with paper caps and rubber bands; two sterilized capillary pipettes in guard tubes; one rubber nipple; 1 oz. each of Jenner's stain, Sterling's gentian-violet-anilin stain, formalin, Gram's iodin solution, 94 or 95 per cent. alcohol, carbol-fuchsin (diluted 1 to 10); *dilute* gentian-violet solution—5 c.c. of a saturated alcoholic solution plus 95 c.c. of distilled water; 100 c.c. of a 20 per cent. aqueous solution of copper sulphate. Supply also a steam sterilizer for final sterilization of the sputum containers and other contaminated apparatus. Bunsen burners should be ready for use.

Directions to the Student.—Examine the patient's sputum with the naked eye; note its rusty appearance in places and its viscidity; the container can frequently be inverted without spilling a drop of the contents. With the forceps supplied, separate a bit of the sputum from the main mass and prepare four smears on slides, using your nichrome wire or a tooth-pick, which latter should be burned immediately after use. Dry the specimens by beating the

Bunsen flame and mark them *a*, *b*, *c* and *d*. Before proceeding to stain these, every student should add to one tube of the meat infusion broth 0.25 c.c., approximately 4 or 5 drops, of both the sterile dextrose solution and the defibrinated blood that has been supplied to his group. Then transfer a piece of the sputum, about the size of a bean, to one of the tumblers containing sterile saline; wash it well by floating it about; transfer it to the second tumbler; repeat the washing there and finally place it in the culture tube containing the dextrose-blood-meat infusion broth (Avery's so-called "artificial mouse"). Label the test-tube with your name and date and place it in the incubator—in a tumbler with some cotton at the bottom, to prevent breakage. Remove it from the incubator the following morning and examine the culture in the afternoon—in actual practice five to six hours incubation is sufficient. Now return to your sputum smears and proceed as follows: Stain (*a*) with Jenner's stain for about three minutes; wash off; allow to dry in the air. Stain (*b*) for one-half to one minute with Sterling's gentian-violet-anilin stain; pour off the excess of stain and cover the slide with Gram's iodin solution; after two or three minutes pour off the iodin solution and replace it with two or three changes of alcohol,—two to three minutes, until no more stain can be extracted in this manner; counterstain with dilute carbol-fuchsin solution, wash off, dry by blotting or beating the Bunsen flame. Flood (*c*) with formalin for three to five minutes; wash off with water; stain with Sterling's solution for one-half to one minute and continue as with specimen (*b*). Stain specimen (*d*) for a few seconds with the *dilute* gentian-violet solution, which has been thus marked, staining the preparation for a few seconds; wash off with 20 per cent. copper sulphate solution; blot (do not wash). Examine all four specimens with the oil-immersion lens. In specimen (*a*) note the character of the cellular elements and the presence of large numbers of end-to-end diplococci, which often appear surrounded by a little colorless halo. In specimen (*b*) you will observe that the organisms have retained the gentian-violet stain and are hence Gram-positive. The treatment of (*c*) and (*d*) was intended to bring out the presence of capsules; the methods employed were those of Wadsworth and Hiss respectively. With the former the capsule appears colorless; with the latter a faint blue. The organisms in question are pneumococci. Draw representative fields.

Answer the Following Questions in Writing.—What organism is the most constant cause of acute croupous or lobar pneumonia?

To what is the rusty color of pneumonic sputum due? What are the essential morphological characteristics of the pneumococcus? Is it a hemolyzing organism? Are pneumococci found in the throats of healthy individuals who have not been in contact with pneumonia cases. What is the essential difference between such organisms and those found in the sputum of the majority of pneumonia patients? What is meant by type pneumococci? How many types are there? What percentage of pneumonia cases are due to these types individually? What is the corresponding mortality? Why is it necessary to wash the sputum before culturing it? What is the object of typing pneumococci in concrete cases? How would you distinguish between streptococci occurring in diplo-form and pneumococci, aside from the presence or absence of a capsule?

Reading.—

Lesson 21.—*Material, Apparatus and Reagents Required.*—The cultures made in Avery's medium, on the day preceding, should be removed from the incubator in the morning and kept in the refrigerator or at room temperature until the class meets in the afternoon. For every six men provide the following: 10 c.c. of antipneumococcus serum, corresponding to the three types, diluted according to their titer; 5 c.c. of sterile ox bile; a basin containing 10 per cent. lysol.

Every man individually should be furnished with a dozen sterilized agglutination tubes, closed with cotton stoppers; corresponding racks; half a dozen sterile capillary pipettes in guard tubes, with a couple of nipples; one short sterile test-tube of ordinary size; a wax pencil.

Supply also a steam sterilizer to disinfect contaminated material at the end of the lesson; an ordinary incubator kept at 37° C., and a high-speed centrifuge. In addition supply the same reagents, used for capsule staining, that were employed the day before.

Directions to the Student.—Prepare a smear from your culture, using your nichrome wire loop; dry and stain it according to Hiss's or Wadsworth's method (see preceding lesson). Examine with the oil-immersion lens. If the culture is a fairly pure one, of capsulated diplococci, centrifugalize for a couple of minutes at low speed to throw down the red cells. Transfer the supernatant fluid to a sterile test-tube, using one of your capillary pipettes.

Experiment A (*Agglutination Test*).—Charge three agglutination tubes marked I, II and III, respectively with approximately 0.5 c.c., 8 drops, of the culture, and add a corresponding quantity of the

respective antisera, I, II and III, taking care to thoroughly mix the two components. Place the tubes in the incubator at 37° C. and examine them at intervals of half an hour. If the diplococcus in question was a type organism agglutination, visible with the naked eye, will be noticeable after one to two hours' standing. In any case reëxamine the next day, meanwhile allowing the tubes to stand at room temperature. If the organism is not a type organism, but belongs to group IV, the tubes will appear equally turbid and no clearing will have taken place. Sketch the final result.

EXPERIMENT B (*Precipitin Test*).—While the tubes charged, as directed above, are in the incubator, add 1 c.c. of sterile ox bile to the remainder of your culture, and incubate for twenty minutes. If the culture was pure the turbidity will clear up owing to the solvent action of the bile upon the pneumococci. If the turbidity has not cleared up entirely, centrifugalize at high speed, until the fluid is perfectly clear. Now charge three agglutination tubes marked I, II and III with 0.5 c.c., 8 drops, of the clear fluid, and add a similar quantity of the corresponding antisera as directed in experiment A. The results may be read in a few minutes and are sharp and distinct; the occurrence of a turbidity in one of the tubes indicates that you are dealing with a type organism, the number of the type corresponding to the number of the antiserum. No turbidity will develop, if the organism in question belongs to group IV. Sketch the final result. Sterilize all contaminated material in the steam sterilizer, at the end of the lesson.

Answer the Following Questions in Writing.—What is the technical term that is applied to the substance in the antiserum which causes the agglutination of the corresponding organisms? What is meant by the term precipitin? What does it precipitate? What is the effect of bile or bile salts upon pneumococci? How would you secure a specimen of sputum, actually coming from the lungs, in a case of pneumonia? If you had reason to believe that your specimen was contaminated by mouth organisms, but that organisms from the lung were also present, how would you proceed? In what other manner could you secure a representative culture, if sputum should not be available? Having determined in a given case, to which group the organism belongs, what would be the corresponding prognosis? What indication for treatment would the examination afford?

Reading.—

Lesson 22.—*Material, Apparatus and Reagents Required.*—For every six students provide 2 oz. of the following stains: a freshly prepared mixture of Neisser's methylene-blue and Neisser's crystal violet, 2 parts of the first and 1 of the latter; Neisser's chrysoidin or Bismarck-brown solution; Loeffler's blue solution; Sterling's gentian violet-anilin solution; Gram's iodin solution; 94 per cent. alcohol; carbol fuchsin solution and Hiss's capsule stain solutions (see Lesson 20); cultures of the diphtheria bacillus on Loeffler's medium; of the Streptococcus hemolyticus, Streptococcus viridans and the pneumococcus on human blood-agar plates and of the meningococcus on a serum plate; 20 c.c. of a sterile, rich emulsion of human blood in dextrose broth.

Furnish every student with three small sterilized cotton swabs, two on straight reeds, one on a wire bent near the end to an angle of about 145°; a wooden tongue depressor; two tubes of Loeffler's blood serum; one tube of about 10 to 15 c.c. of agar; one sterile capillary pipette with nipple; one sterile Petri dish; one plate of dextrose serum agar, 0.4 per cent acid to phenolphthalein; a nichrome wire and a wax pencil. Have available also staining trays, paper napkins, tumblers, cotton, an incubator at 37° C., basins containing 10 per cent. lysol solution; a steam sterilizer to disinfect all contaminated material at the end of the lesson.

Directions to the Student.—Put approximately 3 c.c. of the emulsion of human blood in your Petri dish, melt the tube of agar, furnished you, by heating in the free flame of the Bunsen burner; be careful not to crack the tube; when entirely liquefied, allow it to cool for a few minutes, until you can just handle the heated portion of the tube; flame the mouth of the tube and pour the contents into the Petri dish containing the blood; mix the two well by circular rotation; then set aside to harden for half an hour. While this is taking place study the appearance of the cultures furnished your group, with the naked eye; compare the different types; make a sketch illustrating the difference in the type of hemolysis, shown by the two varieties of the streptococcus and the pneumococcus. Note (*a*) the narrow greenish zone of discoloration of the latter; (*b*) the green color and absence of hemolysis of the viridans colonies; (*c*) the distinct hemolytic area, measuring 2 or 3 mm., surrounding those of the Streptococcus hemolyticus. Now make two smears of each one of the different organisms, in drops of water; dry them by beating the Bunsen flame, and mark them Da and Db, Va and Vb, Ha and Hb, Ma and Mb, Pa and

Pb (D = B. diphtheriæ, V = S. viridans, H = S. hemolyticus, M = meningococcus, P = pneumococcus). Stain Da for ten to fifteen seconds with the Neisser blue mixture; wash off; counterstain for an equal length of time with Neisser's chrysoidin solution or with Bismarck brown; wash off; dry the reverse side with a paper napkin; then beat the flame, until dry. Stain Db for five to ten minutes with Loeffler's blue; wash off and dry as just directed. Stain Va, Ha, Ma and Pa according to Gram, and Vb, Hb, Mb and Pb with Hiss's capsule stain, as directed in Lesson 20. Now examine each slide with the oil-immersion lens and sketch representative groups of the organisms, indicating what each sketch is intended to show. In Da note particularly the polar bodies and their arrangement; in Db the form of the organisms. Compare the form and absence of capsules in Va, Vb, Ha and Hb, Ma and Mb, as compared with Pa and Pb. Note that the streptococci and the pnemococci are "end-to-end" organisms and Gram-positive, whereas the meningococci show a "side-to-side" grouping and are Gram-negative. After completing your microscopic work, make cultures from your working partner's throat and nose on the culture media furnished as follows: Hold down the tongue with the wooden depressor; apply one of the straight reed-swabs to the tonsils, if possible, gently entering the large anterior crypt, and now smear one of the Loeffler medium slants; mark this T. Take a second reed-swab and enter the nose, directing the reed straight backward, not upward; gently press it back to the posterior portion and now smear the second Loeffler slant with the material obtained; mark the tube N.

With your third swab attempt to reach the nasopharynx, behind the velum of the palate and then smear both the serum-agar plate and the blood-agar plate; mark both NP and with the name of the subject. Set your tubes in a tumbler, on cotton, and mark the tumbler with your partner's name. Incubate both tubes and plates at 37° C. until the following afternoon, when they are examined as directed in Lesson 23.

Answer the Following Questions in Writing.—Are diphtheria bacilli only found in the throat of patients suffering from diphtheria, or convalescents from the disease? What is meant by active and what by passive carriers? Why should an examination of the nose of an individual who has recently passed through the disease or who has been in contact with the malady be invariably made, in addition to an examination of the throat, before concluding that he is not a carrier? How would you determine whether the organisms are

virulent? If you should meet with a patient presenting a suspicious-looking exudate in his throat, how could you secure a satisfactory culture if the usual media should not be at hand? What other organisms, besides the diphtheria bacillus, may give rise to the formation of exudates about the tonsils? What is Vincent's angina and to what organism is it due? Name the most important lesions which are produced by the Streptococcus viridans and the Streptococcus hemolyticus respectively? What type of streptococcus is usually associated with septic sore-throat? Are any of these organisms ever met with in the throats of individuals who are, at the time, not suffering from the corresponding infections; is there any ground for the belief that such individuals may act as carriers? What infections seem to predispose to the pathogenic activity of the Streptococcus hemolyticus? Given an outbreak of streptococcus infection, in the surgical division of a hospital, how would you proceed to trace its origin and to prevent its further spread?

Reading.—

Lesson 23.—*Material, Apparatus and Reagents Required.*—The tubes and plates inoculated by the students from each other's throats and noses, during the preceding lesson, after twelve to twenty-four hours' incubation,

For every six students furnish also a living meningococcus culture and the same stains as in the preceding lesson.

Supply each student with one tube of veal infusion broth, one tube of Hiss's serum water inulin and four agglutination tubes, each containing 1 c.c. of Olitsky's medium.

Have available lysol basins and a steam sterilizer.

Directions to the Student.—Examine your cultures on the Loeffler medium for diphtheria bacilli, using Neisser's stain. If none are found, stain smears with dilute carbol fuchsin, for a minute or so, and try to ascertain the character of the organisms that have grown out, on the basis of their morphology. Sketch what you see. Then examine your blood-agar plates, with the naked eye, for the presence of greenish or hemolyzing colonies; fish individual colonies, make smears and stain (*a*) according to Gram, using Sterling's gentian-violet anilin solution, and (*b*) for capsules, using Hiss's method. Having identified colonies of the Streptococcus hemolyticus, so far as this is possible by microscopic methods, inoculate a tube of veal infusion broth with a carefully fished colony; mark it and incubate for twenty-four hours; examine its hemolytic action the next day in

the test-tube experiment and ascertain whether the organism is bile-soluble or not. Sketch the microscopic appearance of the organisms which you regard as streptococci. Inoculate one tube of Hiss's serum water—inulin medium with a colony; observe this daily for three or four days. Now examine your serum-agar plate, at first macroscopically; then fish colonies and see whether any of them are composed of Gram-negative organisms, and present a "side-to-side" grouping. If so, fish suspicious-looking colonies from the plate and transfer to tubes of Olitsky's medium, one colony to a tube; mark the tubes and the corresponding colonies on your plate and incubate overnight. Finally, inoculate one tube of Olitsky's medium with a loopful of living meningococci to serve as a positive control, and place this in the incubator.

Answer the Following Questions in Writing.—What is the action of bile or bile salts on streptococci? what is it upon pneumococci? What is the action of streptococci upon Hiss's serum water inulin medium as compared with that of pneumococci? Is chain formation typical of streptococci? What is the principle underlying Olitsky's method of identifying the meningococcus? What is meant by the normal horse-serum negatives? Name those which are likely to be encountered in the nasopharynx. What is the behavior of the horse-serum negatives toward staining by Gram? With what organism, aside from the horse-serum negatives, is the meningococcus most likely to be confused? How do you finally distinguish between the two when using Olitsky's method?

Reading.—

Lesson 24.—*Material, Apparatus and Reagents Required.*—The cultures made during the preceding lesson.

For every six men provide 20 c.c. of a freshly prepared 5 per cent. emulsion of washed sheep cells; 2 c.c. of sterile ox bile or 2.5 c.c. of a sterile 10 per cent. solution of sodium taurocholate; 2 c.c. of a sterile 10 per cent. dilution of a polyvalent antimeningococcus serum, of high titer, in 0.85 per cent. saline.

Furnish every student with three or four sterile plugged agglutination tubes, the same number of sterile capillary pipettes and a couple of rubber nipples. Provide lysol basins and steam sterilizers.

Directions to the Student.—Transfer 1 c.c. of your streptococcus culture, in veal infusion broth, to one of your agglutination tubes, and 1 c.c. to a second tube; sterilize your pipette by burning it in the flame of the Bunsen burner. Mark your tubes H and B respectively.

To H add an equal volume of the 5 per cent. emulsion of sheep cells, supplied to your group; shake well and incubate for two hours at 37° C., centrifugalize and note whether hemolysis has occurred or not. Sketch the final result, using your red pencil to indicate the occurrence and degree of hemolysis. To B add three drops of ox bile or three drops of the sodium taurocholate solution; sterilize your pipette by heat; mix well and incubate for half an hour at 37° C. Sketch the final result and illustrate at the same time what would have happened if your culture had been one of the pneumococcus. Now study the results which you obtained with your cultures made from the veal infusion agar plate. Set the horse serum negatives, *i. e.*, those tubes in which agglutination has taken place, aside and add to those which are turbid one drop of the antimeningococcus serum supplied to your group. Incubate in a water incubator for two hours and finally make a sketch of your results.

Answer the Following Questions in Writing.—In what portion of the upper respiratory tract is one most liable to encounter meningococci in carriers? How long does the carrier stage usually last? What is meant by a "polyvalent" antiserum?

Reading.—

Lesson 25.—*Material, Apparatus and Reagents Required.*—For every six men provide 4 c.c. of spinal fluid obtained from a case of paresis; the fluid must be free from blood; further, supply the various reagents used for the Wassermann test, in the quantities indicated in Lesson 13, for groups of six men; likewise the necessary glassware —a set for every two men; a water incubator at 37° C. and a few cubic centimeters of a neutral saturated solution of ammonium sulphate.

For every two men also furnish 11 test-tubes of ordinary size, one 2 c.c. pipette, graduated in tenths; half a dozen capillary pipettes, with rubber nipples, and one 5 c.c. pipette—all of which should have been cleansed, as is usual with glassware that is to be used for the Lange colloidal gold test. This set is to be used only for this purpose. In addition, furnish every six students with an ordinary test-tube rack; 25 c.c. of 0.4 per cent. saline made from triply distilled water and 100 c.c. of colloidal gold solution, both in adequately cleansed beakers. Furnish also individual blood-counting chambers.

1. *Directions to the Student* (two men working together).—Take two Wassermann tubes and mark them A and B respectively.

Charge both with 0.2 c.c.—4 drops—of the spinal fluid, furnished your group; add to each one the same quantity of complement (which has already been diluted); A further receives 0.2 c.c. of the diluted antigen and B 0.2 c.c. of saline. Incubate the tubes in the water-bath, or your vest pocket for fifteen minutes; then add to each tube 0.2 c.c. of the sheep cell emulsion and 0.2 c.c. of the diluted amboceptor. Reincubate for fifteen minutes, centrifugalize and note the results. What does the A tube show? what does the B tube show? Is the reaction positive or negative? Sketch the appearances of the tubes.

2. Set up the eleven test-tubes, furnished for the colloidal gold test, and mark them 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11. Using your 2 c.c. pipette, charge tube 1 with 1.8 c.c. of the 0.4 per cent. saline and the remaining tubes with 1 c.c. each. Now add 0.2 c.c.—4 drops—of spinal fluid to tube 1, using a capillary pipette, and breaking this off at a point, so as to furnish a fairly large drop. Take up your 2 c.c. pipette again, blow bubbles into tube 1, so as to thoroughly mix the fluid with the saline; transfer 1 c.c. to tube 2; blow bubbles; transfer 1 c.c. from tube 2 to tube 3, and so on, until tube 10 has been deprived of 1 c.c. of its mixture; this 1 c.c. is discarded. Tube 11 thus only contains saline and serves as a color control in the end. Now add 5 c.c. of the gold solution to each one of the 11 tubes; shake each tube and set the entire lot aside until the end of the period when they should be examined and the color in each noted. Let the tubes stand over night and reëxamine them the next day. The appearance of a bluish tint is noted as 1, lilac as 2 and dark blue as 3, a pale blue as 4 and decolorization as 5, while no change in color is denoted by the mark 0. Express your readings accordingly, in a number made up of 10 numerals. Sketch the result, using colored pencils.

3. *Directions to the Individual Student.*—Place 5 drops of the saturated solution of ammonium sulphate, supplied to your group, in one agglutination tube, add 5 drops of spinal fluid, and note whether a turbid ring appears at the zone of contact of the two fluids, either immediately or on standing for a few minutes (Ross-Jones test). Normal fluid will not give a turbidity. Sketch the result.

Mount a drop of the undiluted spinal fluid on the platform of your blood counting chamber; adjust the cover-glass; let stand for a few minutes; then count the number of leukocytes in 100 large squares using the middle power and taking care not to confound red cells that may be present with leukocytes. Try to ascertain whether the

leukocytes in question are polynuclears or mononuclears; calculate the number for 1 c.mm. of the fluid and give this in your report.

Answer the Following Questions in Writing.—What is the normal cell count of the cerebrospinal fluid? Under what pathological conditions would you expect an increase? What does the Ross-Jones reaction indicate? Under what pathological conditions is it obtained? What is meant by the term a "colloidal" solution of gold? What happens to this solution when a change in color tending toward blue occurs upon mixing it with diluted cerebrospinal fluid—in a certain concentration—obtained under certain pathological conditions? Why is it necessary to use doubly or triply distilled water in making up the reagents, and why is it necessary to cleanse the glassware so thoroughly before use? What constitutes the "paretic curve?" Under what pathological conditions would you expect a positive Wassermann in the cerebrospinal fluid?

Reading.—

Lesson 26.—*Material, Glassware and Reagents Required.*—Furnish every student with a cerebrospinal cell smear, obtained (*a*) from a normal individual, or at any rate from one who has no disease of the meninges or the central nervous system; (*b*) from a case of tabes; (*c*) from a case of paresis; (*d*) from a case of acute anterior poliomyelitis; (*e*) from a case of epidemic meningitis; (*f*) from one of tubercular meningitis; (*g*) from one of pneumonic meningitis-pneumococcus or streptococcus type. All specimens should be stained with methylene-blue.

Furnish also a double set of unstained smears from a case of gonorrheal urethritis, and the necessary reagents to stain by Gram—a set for every six men; supply staining trays and wash bottles.

Directions to the Student.—Examine each slide, first with the low-power—to get a general idea of the number of cells present and their location; then with the oil-immersion lens; note the various types of cells that may be present—small mononuclear leukocytes, polymorphonuclear neutrophiles and endothelial cells. In each slide ascertain the percentage number of the different types and note that in the pyogenic infections the polymorphonuclears predominate, while in the others the small monos control the picture. In the two cases of pyogenic meningitis search for the corresponding organisms—note their morphology and the occurrence or non-occurrence of phagocytosis. Sketch the various pictures and append appropriate legends. Now stain one of the smears of gonorrheal urethritis with

the gentian-anilin solution, twenty to thirty seconds and the other according to Gram (see lesson). Then examine both specimens with the oil-immersion lens; note the morphology of the gonococci, the relative number contained within cells, as compared with those lying free, and the behavior toward Gram staining. Sketch the appearances.

Answer the Following Questions in Writing.—Given a case of pyogenic meningitis, how would you determine whether you are dealing with a meningococcus or a pneumococcus infection, aside from your microscopical findings? Given a case of suspected tubercular meningitis describe the technic which you would employ to ascertain whether this diagnosis is correct?

Reading.—

Lesson 27.—*Material, Apparatus and Reagents Required.*—(*a*) 2000 c.c. of a normal digestive mixture, containing 0.365 per cent. of hydrochloric acid, half a teaspoonful of pepsin powder, and a couple of slices of bread; the mixture should be incubated for an hour at 37° C. before class work begins; this mixture should be marked in such a manner as not to indicate its composition to the student. (*b*) 2000 c.c. of a 1 per cent. solution of lactic acid and a couple of slices of bread. This mixture also should be incubated for one hour and marked. For every six students provide 350 c.c. of the above two digestive mixtures; 200 c.c. of $\frac{n}{10}$ sodium hydrate solution; 25 to 30 c.c., each of a 0.5 per cent. alcoholic solution of dimethyl-amino-azo-benzol; 25 to 30 c.c. of a 1 per cent. aqueous solution of alizarin-mono-sulphonate of sodium; 25 to 30 c.c. of a 1 per cent. alcoholic solution of phenolphthalein; 25 to 30 c.c. of a saturated aqueous solution of the sesquichloride of iron; 25 to 30 c.c. of a concentrated solution of carbolic acid; one eighth of the white of a hard boiled egg; 100 c.c. of milk.

Every student should be furnished with: a Mohr burette with stand; five whiskey glasses or beakers of corresponding size; a stirring rod; half a dozen ordinary test-tubes and a 5 c.c. pipette.

Directions to the Student.—Provide yourself with 50 c.c. each of the two digestive mixtures; mark them to correspond to the stock mixtures furnished your group; fill your burette to the 30 c.c. mark with $\frac{n}{10}$ sodium hydrate solution; be sure that there are no bubbles in the rubber cuff. Measure off 5 c.c. of mixture A—unfiltered—into each one of your three whiskey glasses and mark these P, A and D respectively. To P add one or two drops of the phenolphthalein

solution as indicator; to A three or four drops of the alizarin solution and to D three or four drops of the dimethyl-amino-azo-benzol solution. Take your burette reading; lower meniscus. Place a sheet of white paper beneath the burette and place the glass P upon this. While stirring with your glass rod allow the $\frac{n}{10}$ solution to flow into your mixture until a permanent pink color results; as soon as this point has been reached take your reading again; the number of cubic centimeters of the $\frac{n}{10}$ alkali used, when multiplied by 20, will give you your total acidity. Then titrate A until a pure violet is obtained; the difference in your readings when multiplied by 20, will give you the acidity due to free acids and acid salts. Finally titrate D until the last trace of red has disappeared and the color has turned a greenish yellow; the difference in your readings, multiplied by 20, will give you the acidity due to free hydrochloric acid. Note your findings in tabulary form, as shown below:

Total acidity	—
Free HCl	—
Combined HCl	—
Acid salts and organic acids	—

Next fill a test-tube with water; add one drop of the sesquichloride of iron solution; mix the two and dilute still further, until the solution is almost colorless; pour one-half of the contents of the tube into a second tube and use one for comparison; now add a few cubic centimeters of the digestive mixture to one of the tubes, and note whether or not a canary yellow color results—presence or absence of lactic acid (Kelling's test). To ascertain this point, test also, according to Uffelmann's method, without previous extraction by means of ether: In a test-tube mix 3 drops of your chloride of iron solution with 3 drops of the concentrated carbolic acid solution; dilute with water until an amethyst blue color results. To this mixture add a few cubic centimeters of the digestive mixture, when in the presence of lactic acid a canary yellow color will result.

Now place approximately 15 c.c. of the digestive mixture in a test-tube; add a tiny slice of coagulated egg and incubate overnight. If pepsin is present the egg will be digested and undergo solution.

Finally treat 5 to 10 c.c. of milk, in a test-tube, with 3 to 5 drops of the digestive mixture and incubate for ten to fifteen minutes at 37° C. If coagulation occurs during this time it may be inferred that the milk curdling ferment was present. Arrange your findings in tabulary form below your acidity determinations. After having completed the examination of the A mixture, repeat the entire proc-

ess with the B mixture, and arrange your findings in a corresponding manner.

Answer the Following Questions in Writing.—What is meant by free HCl? What by combined HCl? Define (*a*) euchlorhydria, (*b*) hyperchlorhydria, (*c*) anachlorhydria, (*d*) hyperacidity, (*e*) anacidity. What is meant by gastric hypersecretion? what by achylia gastrica? Under what pathological conditions is lactic acid in notable quantity usually found? Under what pathological conditions does anachlorhydria occur? Why is it necessary to obtain the gastric contents at a certain time after the administration of a meal of known composition? What is the idea underlying the fractional analysis of the stomach contents? Describe in a general way the role which an indicator plays in volumetric analysis, using the titration of a normal digestive mixture in the presence of dimethyl-amino-azo-benzol on the one hand and phenolphthalein on the other, as an example.

Reading.—

Lesson 28.—*Material, Apparatus and Reagents Required.*—For every six men furnish 2 c.c. of stomach contents, containing Boas-Oppler bacilli; a similar quantity of stomach contents containing sarcinæ. The stomach contents should be procured the day before and allowed to stand in the ice-box overnight; the next morning the supernatant fluid should be carefully decanted from the sediment and the latter transferred to suitable little bottles and layered with toluol; thus preserved the material will keep for many months. Mark the bottles "Oppler-Boas bacilli" and "Sarcinæ" respectively.

For every six men furnish also small samples of moderately thin emulsions (*a*) of normal feces, (*b*) of a fatty stool, (*c*) of feces obtained from an acute enteritis or colitis. Also supply a somewhat larger sample—5 to 10 grams—of a stool containing occult blood; mark this "to be examined for occult blood."

Every six students should be furnished with 15 to 25 c.c. of alkaline phenolphthalin solution; a few cubic centimeters of a 10 per cent. solution of hydrogen peroxide; 0.5 gram of powdered benzidin; 10 to 15 c.c. of glacial acetic acid; 10 c.c. of a 3 per cent. solution of hydrogen peroxide; 2 oz. of a 1 per cent. aqueous solution of methylene-blue; 1 oz. of a saturated alcoholic solution (70 per cent.) of Sudan III; 1 oz. of Lugol's solution.

Furnish every student with a few ordinary test-tubes, a small piece of filter paper, slides and cover-glasses.

Directions to the Student.—First examine the fecal specimen marked "for occult blood," using (*a*) the phenolphthalin test and (*b*) the dry benzidin test. (*a*) In a test-tube emulsify a bit of the fecal material—the size of a pea—in 2 c.c. of distilled water—or at any rate in water which itself does not give the reaction,—add 1 c.c., approximately, of the phenolphthalein solution and one or at most two drops of the 10 per cent. solution of hydrogen peroxide. In the presence of blood a pink color will develop, either at once or on standing for a few minutes, the rapidity of its appearance and its intensity varying with the quantity present. (*b*) Smear a tiny bit of fecal material—the size of a match-head—over a piece of white filter paper and drop on this 2 or 3 drops of benzidin solution. Prepare the latter yourself as follows: In a small test-tube place a little powdered benzidin, about twice the size of an ordinary pin-head; add 1 c.c.—15 drops—of glacial acetic acid and 10 drops of a 3 per cent. solution of hydrogen peroxide. In the presence of blood-coloring matter a greenish-blue color will develop on the filter paper in a few seconds. Next mount a droplet of the emulsified fecal material that you have been testing; adjust a cover-glass and examine with the middle power for red blood cells. Note that none can be demonstrated, the blood being occult. Now prepare a smear from the specimens of gastric contents, marked "Oppler-Boas bacilli" and "Sarcinæ" respectively; dry by beating the flame of the Bunsen burner and stain for thirty seconds with methylene-blue; wash off, dry and examine with the oil-immersion lens. The Oppler-Boas bacilli will appear as long, stout rods, occurring either individually or in chains. In the same specimen deeply stained yeast cells will also be found; look for budding forms. In the "Sarcinæ" specimen look for the characteristic cotton-bale like packets. Make sketches of your findings. Next mount a small drop of the emulsified "normal" fecal material; adjust a cover-glass and examine with both middle and low power. Note that the entire "background" of the picture is made up of countless numbers of bacteria of various kinds, bacilli and cocci, either undergoing active or passive movement. With the low power search (*a*) for muscle fibers—these appear as yellow or yellowish-brownish cast-like formations, with parallel borders; with the higher power the transverse striation can usually be made out. Note the relative number, bearing in mind that the stool is from a normal individual. With the low power search (*b*) for undigested vegetable matter—spirals and cells which may or may not contain starch—the latter can be demonstrated

by allowing a drop of Lugol's solution, added from the side, to mix with the specimen, when all starchy material will turn a bluish black; (*c*) look for vegetable hairs, presenting a central canal, either broken off or starting from a broad base; these are often mistaken for worms by the beginner. Draw these various appearances, and append corresponding legends. Now mount a drop of the specimen marked "fatty stool;" adjust a cover-glass and examine with the middle power. Note the enormous number of fatty acid crystals, occurring individually and in sheaves of various sizes; look also for fat globules. Allow a drop of the Sudan III solution to flow under the cover-glass, from the side, and note that the fat globules are colored by the dye. Draw. Finally, mount a drop of the "enteric" stool; adjust a cover-glass and examine with the middle power. Note the appearance of the admixed mucus and the adhering cells, which are, for the most part, desquamated columnar epithelial cells, characterized especially by the irregularity of their form; search also for pus corpuscles and red blood cells—note the small number of the former. Draw.

Answer the Following Questions in Writing.—What organism is largely responsible for the production of lactic acid in the stomach contents of patients suffering from cancer of the stomach? Under what conditions are sarcinæ liable to occur in the stomach contents? How is phenolphthalin formed from phenolphthalein? What happens to the phenolphthalin when it is brought in contact with occult blood in the presence of hydrogen peroxide? Why does the solution turn pink? What happens to the benzidin under like conditions? What is meant by lientery; what by steatorrhea? Why will the latter condition result when the common bile duct is obstructed? Under what pathological conditions does mucus occur in the stool in large amounts? What is an enterolith? What is the most common bacterium occurring in the feces?

Reading.—

Lesson 29.—*Material Required.*—Furnish every student with a blood smear or blood smears showing (A) the parasite of benign tertian fever (*a*) in the early stage of its development, (*b*) forms twenty-four hours old, (*c*) mature forms; (B) the parasite of quartan fever, also if possible in various stages of its development; (C) the parasite of malignant tertian fever (*a*) in the early stage of its development, (*b*) presenting crescentic forms. Furnish also smears from the spleen or bone-marrow of a patient dead with malignant tertian

fever to show adult schizonts. The specimens should all be stained with an eosin-methylene-azure mixture.

Directions to the Student.—The smears should be studied with the oil-immersion lens; crescents may be searched for with the low power. Note that the body of the malarial parasite is colored blue while its chromatin appears red. In every cell determine whether the chromatin is present in a single compact mass or whether it shows evidence of division—a distributed nucleus. Note the melanin granules—their color and number—in the more mature organisms. Observe the occurrence of Schüffner's dots—azurophilic granules—in the bodies of the red cells, harboring the parasite of benign tertian fever. Note that the red cells infected with plasmodium vivax tend to increase in size, while this is not the case in quartan and malignant tertian fever. Observe the signet-ring appearance of the young estivo-autumnal forms. Sketch representative cells showing these points and also the process of aging from the young to the adult form. In each one of your specimens search for male and female gametes; draw such cells and place alongside of them the corresponding schizonts.

Finally, do a differential leukocyte count in one of your benign tertian, quartan and malignant tertian specimens, paying particular attention to the correct differentiation of the large mononuclears, as compared with the small mononuclear forms.

Answer the Following Questions in Writing.—Why is an eosin-methylene azure mixture preferable to an eosin-methylene-blue mixture for staining the malarial parasite? To what class of protozoa does the malarial organism belong? to what subclass? to what order? to what suborder? to what family?

Write the Latin name of each one of the three types of the malarial organism that you have studied; give the generic and the species name. By whom was the malarial organism first discovered? What is meant by schizogony? What term is applied to the products of schizogony? What is a schizont? What is meant by sporogony? How are its products termed? What is meant by gametes? What is the difference between a gamete and a gametocyte? What does the prefix macro and micro denote in connection with the term gamete or gametocyte? What is meant by the term zygote? How many hours does the benign and malignant tertian parasite require for its cycle of development? What is the most striking point in the leukocytic formula of a case of malarial fever? Prepare a table showing the differences between the three types of organisms.

Reading.—

Lesson 30.—*Material Required.*—Furnish each student (*a*) with a blood smear from a rat that has been infected with Trypanosoma lewisi or T. gambiense; (*b*) with a smear from a culture of Leishmania infantum; both should be stained with an eosin-methylene-azure mixture; (*c*) furnish every six men with specimens of Anopheles maculipennis, Culex pipiens, Glossina palpalis, Pulex irritans, Cimex rotundatus, Ornithodorus moubata, Conorhinus megistus.

Directions to the Student.—In specimen (*a*) note the morphology of the organism. The posterior end is drawn out in a long, pointed, nose-like projection. The large nucleus is located in the anterior third of the body. The blepharoplast is rod-shaped and stands at an angle to the body of the parasite, the flagellum originates near the latter; it runs forward in the margin of the undulating membrane and then continues by itself. Sketch these various points. Next examine specimen (*b*). Search for non-flagellated and flagellated forms; the latter develop from the former; note the general morphology, the presence of a macronucleus and a blepharoplast. The latter is smaller than the nucleus; it stains more deeply and is placed near the periphery. There is a single flagellum and no undulating membrane. Sketch these various features. Finally examine the various disease-carrying insects which have been furnished your group. Draw the wing of the two types of mosquitoes.

Answer the Following Questions in Writing.—To what class of protozoa do the trypanosomes and Leishmania belong? To what subclass? What pathological conditions in men are caused by the former and what by the latter? By what insects are these various infections carried? Arrange your answers in tabulary form: Name of disease; causative parasite; transmitting insect. Make a diagrammatic sketch of the life-cycle of the malarial organism. By what organism is relapsing fever produced? By what insect is the disease transmitted? Name the various spirochetes which are pathogenic for man, each with the corresponding disease that it causes.

Reading.—

Lesson 31.—*Material Required.*—If a case of amebic dysentery is available fresh fecal material, kept at body temperature, should be supplied—a small portion for every six men. If such material is not available, closely related organisms may be secured from the following sources: (*a*) Procure some of the ooze from the bottom of a pond or slowly moving stream; place it in wide-mouthed bottles; cover with a fair amount of water; add a few cubic centimeters of plain broth and

allow the mixture to stand for four or five days, at room temperature. Amebæ will then be found in abundance in the scum that has formed at the surface of the water, and can be cultured on Musgrave and Clegg's agar. (*b*) Have available a number of mice—two for every six men—killed with chloroform, tacked up on boards and opened up; in the head of the cecum and the upper portion of the colon of every one out of two animals Entameba muris which closely resembles Entameba coli may be found in small numbers. (*c*) Have ready a number of frogs, pithed and tacked up; in the intestinal contents the Entameba ranarum may be found and serve to give an idea of the morphology of the Entameba histolytica. (*d*) Have available also preserved human feces, containing the Endameba histolytica and Entameba coli in the encysted state; use 10 per cent. formalin in saline as preservative. (*e*) Supply also stained smears of material containing the two types of amebæ. The specimens should be wet—fixed in alcoholic bichloride and stained with Delafield's hematoxylin or Heidenhain or Rosenbusch's iron hematoxylin.

Directions to the Student.—(1) If fresh human feces containing parasitic protozoa are available mount a small drop, emulsified with a little saline if necessary; cover with a cover-glass; ring with vaselin and examine first with the low power to locate the organisms, then with the middle power to study their structure and mode of locomotion. In an amebic dysentery stool the Entameba histolytica will be observed undergoing most active ameboid movements, during which the division of the cytoplasm into a hyaline ectosarc and a granular endosarc can readily be made out. Most of the organisms will be found to carry red cells in their interior. In such stools epithelial cells and bacteria, contrary to what one finds in bacillary dysentery, are not numerous. Do not confound swollen tissue cells containing a degenerating nucleus with quiescent amebæ; if in doubt, warm the slide by laying a heated coin on the slide and do not call the cell an ameba unless you can bring it to put out pseudopodia.

Make careful drawings of moving and quiescent organisms; show the small indistinct ring nucleus, the presence of food vacuoles, containing red cells; the granular endosarc and the hyaline ectosarc in quiescent forms, and the manner in which the pseudopodia are projected. (2) Mount a drop of preserved human fecal material containing encysted amebæ both of the histolytica and the coli variety; note the large size of the latter and the presence of eight nuclei as compared with the four nuclei and the smaller size of the

former. In the histolytica cysts from one to three chromidia can usually be made out, while the cysts of Entameba coli rarely contain chromidia; when present they appear like threads and spicules. Draw. (3) If fresh human material is not available, mount a drop of the surface scum from the culture of the free living type that was supplied to your group; cover with a cover-glass; ring with vaseline and examine as above. Make drawings. (4) Mount a droplet of the contents of a mouse's colon; dilute with a droplet of saline and search for amebæ. The Entameba muris closely resembles the Entameba coli, and its cysts, which may be found in the feces, if the vegetative form occurs in the intestine, like those of the Entameba coli contain eight nuclei. Entameba coli is not as actively motile as Entameba histolytica; its pseudopodia are broader and there is no separation of the cytoplasm into ecto- and endosarcs in the quiescent organism. The few vacuoles contain bacteria but no red cells, and the nucleus is quite distinct. Draw. (5) Mount a drop of the intestinal contents of a frog and search for amebæ resembling the Entameba histolytica. Study their structure and locomotion. Draw. (6) Study the stained preparations and make drawings.

Answer the Following Questions in Writing.—What organism is the cause of chronic dysentery? Who first established the causative role of the organism in question in reference to chronic dysentery and liver abscess? Is the Entameba coli pathogenic? Prepare a table showing the points of difference between the histolytica and the coli (*a*) in the motile, (*b*) in the encysted stage. To what class of protozoa do the amebæ belong? To what subclass? To what order?

Reading.—

Lesson 32.—*Material Required.*—Furnish every six students with a small sample of a freshly voided stool that has been kept at body temperature containing (a) Trichomonas intestinalis, (*b*) Lamblia intestinalis, (*c*) Balantidium coli. If fresh material is not available furnish specimens that have been preserved with formalin. If such material also is not at hand, closely related forms may be procured from the following sources: (*a*) Trichomonads and Lamblias are common in the fresh feces of guinea-pigs, rats and mice; the fecal matter is conveniently emulsified in a little saline. (*b*) Opalina and Nyctotherus cordiformis may be found in the contents of the large intestine of frogs; Nyctotherus ovalis is frequently encountered in

the intestine of the cockroach. (*c*) Paramecium may be obtained from amebic cultures in stagnant water. All such material can be readily preserved with formalin. (*d*) Furnish stained specimens of these various types.

Directions to the Student.—Mount drops of the various materials, emulsified in a little saline if necessary; adjust cover-glasses and ring with vaselin. Examine all the specimens both with the low and the middle power. In the specimens containing trichomonads note their size and motility; the organism is pear-shaped; three flagella are directed forward; the fourth is directed posterially and clinging closely to the body of the organism forms an undulating membrane, which extends to the posterior pole. In the stained preparation note the nucleus. Draw these features. In some specimens of lambliasis cysts only are encountered; these are formed in the large intestine through the union of two individuals. They are oval, the longer diameter measuring about one and a half of a red cell. The organism proper is pear-shaped and provided with four pairs of flagella; the posterior surface is convex; the anterior surface presents a depression in which the mouth opening of the organism—the peristome—is located. In the Balantidium specimen note the large size of the organism which is covered with actively motile cilia which are arranged in longitudinal rows; they are grouped most densely about the funnel-shaped mouth. In the posterior portion are two contractile vacuoles which communicate with each other and open to the outside—the anus. In the stained specimen note the two nuclei, of which the macronucleus is kidney shaped. Draw. Opalina, Nyctotherus and Paramecium are closely related to Balantidium. Opalina ranarum is oval in shape and flattened; it has no cystostome; it contains numerous vesicular nuclei, but no contractile vacuoles; waste material is eliminated through a system of canals. Draw. Paramecium is a long, spindle-shaped organism, provided with a slit like cystostome on one side; it contains a macro- and a micronucleus, and two contractile vacuoles with excretory canals arranged about them in a rosette form. Draw. Nyctotherus cordiformis is kidney-shaped; its body is evenly covered with cilia in long rows; on one side of the long cystostome the cilia are longer and thicker than on the other. There is a kidney-shaped macronucleus with a micronucleus, lying in its concavity. In the posterior end there is a large vacuole which opens to the outside. Draw.

Answer the Following Questions in Writing.—To what class, subclass and order does each one of the following protozoa belong:

Trichomonas intestinalis, Lamblia intestinalis, Balantidium coli? To what class does Opalina, Paramecium and Nyctotherus belong? What is the pathological significance of each? What is the blood picture in uncomplicated cases of amebiasis (histolytica)? What is it in cases complicated with liver abscess?

Reading.—

Lesson 33.—*Material Required.*—Furnish every group of six men with small samples of fecal material, properly emulsified with saline, either fresh or preserved with formalin (10 per cent.), containing the ova of (*a*) Tenia solium; (*b*) Tenia saginata; (*c*) Hymenolepis nana; (*d*) Dibothriocephalus latus; (*e*) Fasciola hepatica; (*f*) Clonorchis endemicus; (*g*) Paragonimus westermani; (*h*) Schistosomum hematobium; (*i*) Schistosomum japonicum.

Directions to the Student.—Mount a drop of each one of the fecal specimens furnished your group; adjust a cover-glass and examine, first with the low power of the microscope, so as to learn to rapidly locate the ova and to form a mental picture of their size, form, color and general appearance. Then examine each specimen with the middle power and make careful drawings.

In the tape-worm specimens note that the ova are sometimes surrounded with an oval vitelline membrane containing yolk spheres.

The eggs of Tenia solium and Tenia saginata are round, of a brownish color and surrounded by a thick, radially striated membrane. In the interior the six hooklets of the embryo-hexacanth or oncosphere—can usually be made out. The ova of Hymenolepis nana are colorless and present two distinct membranes; the inner one has two knobs from which filaments emanate; in the interior the hooklets of the embryo can be made out. The eggs of D. latus are oval; they are enclosed in a brown envelope which is operculated —a little pressure on the cover-glass will cause the operculum to open. The contents consist of protoplasmic spherules, all of about the same size, which are lighter in color at the center than at the periphery. The embryo, which at times escapes from fully developed ova, on pressure, is ciliated and contains six hooklets. The eggs of Fasciola hepatica, Paragonimus westermani and Clonorchis endemicus are brown, oval and operculated; those of Clonorchis are enclosed in a colorless envelope; slight pressure upon the cover-glass will cause the little lid to open. The ova of Schistosomum hematobium are fusiform, yellowish, non-operculated and provided with either a terminal or a lateral spine; their shell is very thin.

The ova of S. japonicum are oval, transparent, but spineless, and in size and general appearance resemble those of the hookworm. When water is added to the fresh specimen the ciliated embryo, which can be made out in the transparent shell, escapes in about ten minutes and swims about quite actively.

Answer the Following Questions in Writing.—To what phylum, class and order does each one of the parasitic worms, whose ova you have just studied, belong? Name the intermediary host of each, through which infection of the human being occurs. Name the human habitat of the adult organism in each.

Reading.—

Lesson 34.—*Material Required.*—Furnish every student with a permanently mounted specimen of a mature segment of T. saginata, and every six to eight students with mounted specimens (*a*) of a mature segment of Tenia solium and Dibothriocephalus latus; (*b*) of heads and upper segments of the three tape-worms in question; (*c*) with entire specimens of Hymenolepis nana and with as many of the various flukes, whose ova were studied in the preceding lesson, as may be available. The gross specimens should be cleared in lactophenol and mounted in glycerin jelly.

Lactophenol is composed of 1 part of pure phenol crystals, c. p., 1 part of lactic acid, 2 parts of glycerin and 1 part of distilled water.

To prepare glycerin jelly take 7 parts of gelatin, allow to soak in 42 parts of distilled water for two hours; add 50 parts of glycerin; heat to boiling on a water-bath while stirring; add 1 part of phenol and finally filter through glass wool.

To mount a specimen warm the jelly until it becomes fluid; place the specimen in a small quantity of the jelly, adjust a cover-glass and after the jelly has coagulated ring with Kroenig's wax. This is prepared as follows: Melt 2 parts of bee's wax in an evaporating dish; slowly add 7 to 9 parts of powdered colophonium (rosin) while stirring and pour into small tin boxes. To ring a specimen heat a piece of wire, bent at a right angle; dip it into the wax and seal the cover-glass along its edge.

Furnish every six men also with small fecal samples—preserved with 10 per cent. formalin in saline, containing the ova of the following parasites: Ascaris lumbricoides, Oxyuris vermicularis, Necator americanus and Trichocephalus trichiurus.

Directions to the Student.—(1) Make a careful drawing of a mature segment of T. saginata. Note the position of the genital pore leading

into the genital sinus, and the vagina running to the uterus which presents from fifteen to thirty slender dichotomous branches; observe also the vas deferens and the testicles scattered throughout the proglottis. The genital pores in T. saginata occur on alternate sides in the different segments. (2) Next, draw a mature segment of T. solium and note the difference in the number and arrangement of the uterine ramifications. (3) With a hand magnifying lens, or the ocular of your microscope inverted, study the difference in the structure of the head of T. solium as compared with T. saginata. Note the general form, the presence or absence of a rostrum, the presence or absence of hooklets and the appearance of the sucking discs. Draw. (4) Study and draw the structure of a segment of Dibothriocephalus latus along the same lines as in the case of T. saginata. The mature segments are broader than long; the genital pores are in the median line. (5) Study and draw the mounted specimen of Hymenolepis nana; examine the head and note the presence of a retractile rostrum surmounted by a circle of hooklets. The genital pores are non-alternating. (6) Make a careful drawing of an adult Fasciola hepatica. Note its leaf-like form, the presence of two suckers—one located on the ventral surface. Between the two is the genital pore which leads into a skein-shaped uterus; note also the greatly branched testicles. Draw. (7) Mount drops of the fecal specimens furnished your group; adjust cover-glasses and examine, first, with your low power and then with your middle power for the presence of the corresponding ova; note their size, color, form and structure and make careful drawings of each type. Look for the presence of Charcot-Leyden crystals. (*a*) The fertilized ova of Ascaris lumbricoides are elliptical and provided with a thick, transparent shell; there is also an external coating which is usually colored a yellowish brown and provided with protuberances giving the entire egg the appearance of an English walnut when viewed with a certain focus. The ovum proper is single-celled. When freshly passed the eggs are colorless. Unfertilized eggs are also frequently seen; these are long and more opaque than the fertilized ones. Sometimes ova are met with which have lost their albuminous outer coating and present a smooth, colorless shell—these are often not recognized as Ascaris eggs by the beginner. (*b*) The ova of Oxyuris are colorless, flattened on one side and provided with a smooth double-contoured shell; they contain an embryo at the time that they are passed in the feces. (*c*) The ova of Necator have an oval form and a smooth surface;

the shell has a double contour; the contents are granular, grayish in color and on careful observation will be seen to be composed of a varying number of segments; in material that has stood for twenty-four to forty-eight hours motile embryos may be seen within the shell. (*d*) The ova of Trichocephalus trichiurus are elliptical in shape, brown in color and provided with a double-contoured shell, with a depression at each end, which is closed by a little knob-like lid. The contents are coarsely granular and contain a single cell at the time that the eggs are passed with the feces.

Answer the Following Questions in Writing.—Name the class and order to which each one of the parasitic worms belong whose ova you have just studied. How does infection with each occur in man? Name the habitat of each in man. What is the most constant feature of the blood picture in the intestinal helminthiases? Where and under what pathological conditions may Charcot-Leyden crystals be encountered aside from the helminthiases? What is meant by a miracidium? What are cercariæ? What symptoms on the part of the patient would lead you to examine the stool for intestinal parasites or their ova?

Reading.—

Lesson 35.—*Material Required.*—1. Furnish every group of six to eight students with as many gross specimens of the following adult parasites as may be available: (*a*) Ascaris lumbricoides—male and female; (*b*) Necator americanus—male and female; (*c*) Trichocephalus trichiurus—male and female; (*d*) Oxyuris vermicularis—male and female; (*e*) Trichinella spiralis—male and female; (*f*) Strongyloides intestinalis—female.

2. Furnish also (*a*) preserved tissue specimens—taken from a dog showing hookworms attached to the intestinal mucosa; (*b*) meat infected with trichinella—trichinous rats; (*c*) sections of muscle tissue showing encysted trichinella embryos; (*d*) fecal material containing the embryonic worms of Strongyloides intestinalis—if possible living.

Directions to the Student.—(1*a*). In examining the specimens of Ascaris use a hand lens or the ocular of your microscope inverted. Note the three oral papillæ, one dorsal and two ventral; the transverse striation and color of the body; the tail end of the male is rolled up on its ventral surface and provided with two spicules which project from the subterminal cloaca. The genital aperture of the female is located directly behind the anterior third of the body.

Draw. (1*b*) Examine the hookworms with the low power of the microscope. Note their size. At the opening of the buccal cavity of Necator americanus there will be seen two broad lips and a blunt dorsal conical tooth; beside these there are four buccal lancets. At the tail end of the male will be seen the umbrella-shaped copulatory bursa; the tail end of the female is pointed; the genital aperture of the female is located in the upper half. Draw. (1*c*) Examine the Trichocephalus specimen with a hand lens and the low power. Note the whip-like anterior end of the worm. The tail end of the male is curled in a spiral and presents a single terminal spicule. The female is a little larger than the male; its tail end is not curled upon itself. Draw. (1*d*) Examine the Oxyuris specimens with the low power; the female is the longer; the tail end of the male is bent upon itself and provided with a single spicule; that of the female is straight. In both the bulbous esophagus can be made out. The female is often distended with ova. Draw. (1*e*) Examine the Trichinella specimens with the low power; the female is longer than the male. The esophagus appears as a serrated line. The female has a rounded posterior extremity provided with a slit-like cloaca. The enlargement in the posterior end of the male is the testicle; the male has two tongue-like appendages without a spicule. Draw. (1*f*) Examine the strongyloides specimens with the low power. All the adult worms that are found in the intestinal mucosa are female. Note the pointed, four-lipped mouth and the filariform esophagus. The tail end is sharply pointed; the genital opening is located near the beginning of the lower third of the body. In the uterus a row of ova may be seen which closely resemble those of the hookworm. The embryos develop *in situ* and find their way into the intestine, where the ova are but rarely found—only after active purgation. Draw. (2*a*) Note the firm attachment of the hookworm to the mucosa. (2*b* and *c*) Examine the meat with a hand lens and note the lemon-shaped cysts. Examine the section with the low power; note that the long axis of the cyst is placed in the long axis of the muscle fibre. Each cyst contains one and sometimes two or more larvæ. Note the large number of eosinophilic leukocytes about each cyst. Make careful drawings. (2*d*) Mount drops of the fecal material containing strongyloid embryos; cover with a cover-glass and search for motile larvæ; note their method of progression. Examine one that has come to rest, with the middle power; note the pointed tail and the double esophageal bulb (rhabditiform larvæ). Make drawings.

Answer the Following Questions in Writing.—How soon after infection with trichinella would you expect to find the larvæ in the blood? How soon would you be apt to find them in the muscles? Which muscles are usually the most heavily infected? How does infection of the human being take place? What role does the rat play in the dissemination of the disease? What organism is responsible for so-called Cochin-China diarrhea? In what parts of the United States is the organism also met with?

Reading.—

Lesson 36.—*Material, Apparatus and Reagents Required.*—Supply every six men with 600 c.c. of an albuminous urine—marked A—and 600 c.c. of a urine containing sugar, acetone and diacetic acid—marked B. The urine should have been preserved with chloroform. Furnish about 50 c.c. also of a urine containing bile pigment; mark this C—to be examined for bile pigment only.

Of reagents furnish every six students with 6 oz. of concentrated nitric acid; 1 oz. of a saturated solution of sodium chloride; 1 oz. of 5 per cent. acetic acid; 1 oz. of a 10 per cent. solution of potassium ferrocyanide; 6 oz. of Fehling's solution No. 1 and 6 oz. of Fehling's solution No. 2; 6 oz. of Nylander's solution; half a cake of bakers' yeast; 1 oz. of tincture of iodin, diluted 1 to 10 with alcohol; a few grams of sodium nitroprusside; 4 oz. of a 10 per cent. solution of ferric chloride; 6 oz. of 20 to 30 per cent. sodium hydrate solution; 6 oz. of glacial acetic acid; 6 oz. of Obermeyer's reagent; 2 oz. of a 5 per cent. solution of silver nitrate; 4 oz. of chloroform.

Furnish every individual student with the following: 1, 2 or 3 oz. conical glass; a few pieces of filter paper and a funnel; a dozen test-tubes of ordinary size; a couple of agglutination tubes; a test-tube rack; a couple of 4 oz. beakers; a urinometer; a 100 c.c. cylinder; red and blue litmus paper and four agglutination tubes.

Directions to the Student.—From the two samples of urine furnished your group take a specimen of 100 c.c. each in your two beakers and mark them A and B, to correspond to the stock samples. Examine each specimen according to the following plan: Note the color; is it light, medium or dark amber; or is it brownish or reddish in color; test the reaction with litmus paper; is it acid, alkaline or amphoteric. Take the specific gravity; do not drop the urinometer into the urine, but immerse it with care and see to it that it is dry before being used.

(1) Next, test for albumin, using each one of the following three

tests: (*a*) The cold nitric acid test. Place 10 to 15 c.c. of urine in the conical glass; incline the glass and allow concentrated nitric acid to flow down its side; being heavier than the urine it will sink below the latter; along the line of contact a white turbid ring will form, if albumin be present—either immediately or on standing for a few minutes; the density and the width of the ring will be proportionate to the amount of albumin present. On standing a white ring will also form higher up than the line of contact, in the clear urine; this is due to the precipitation of urates. (*b*) If albumin has been demonstrated with the above test, place 10 to 15 c.c. (1 to 1½ inches) of the same urine in a test-tube; add a few drops (3 to 10) of 5 per cent. acetic acid, so as to make the specimen markedly acid; then add approximately one-sixth of its volume of a saturated solution of sodium chloride and carefully bring to the boiling-point; if albumin is present a turbidity will result, and if the amount be larger than a trace, the substance will flake out in flocculent form. (*c*) Take another sample of the same urine; acidify strongly with acetic acid and add a few drops of 10 per cent. potassium ferrocyanide solution; if albumin is present a turbidity or a flocculent precipitate will develop, according to the amount present.

(2) Now test for sugar, using each one of the following three tests: If albumin was present, acidify the urine with acetic acid, boil and filter. If no albumin was present, boil a little of the urine to remove traces of chloroform, which had been added as a preservative and which would interfere with the tests. Then proceed as follows: (*a*) Place 5 c.c. approximately of Fehling's solution No. 1 in a test-tube; add an equal volume of Fehling's solution No. 2; mix well; dilute with about four volumes of water and bring the upper portion of the mixture to the boil, holding the tube with a manifolded strip of paper; now add about 1 c.c. of urine, warm the mixture for a moment and set the tube aside. In the presence of sugar a yellowish or reddish precipitate will develop either at once or on standing for a few minutes. The development of a greenish color does not indicate sugar. When in doubt apply the fermentation test to the same sample (see below, *c*).

(*b*) In lieu of the above test Nylander's test may be used. Place 5 to 10 c.c. of the urine in a test-tube; add one-tenth the amount of Nylander's solution and bring the mixture to a simmer; keep this up for a couple of minutes. In the presence of sugar a

grayish, dark brown and finally a black precipitate is obtained; the rapidity with which the change in color appears, as well as its intensity, depends upon the quantity of sugar that is present.

(*c*) Take a small bit of yeast; emulsify it in some of the urine to be examined; introduce one of the agglutination tubes, mouth downward into a large test-tube; fill the tube with the yeast-urine mixture; close it with your finger and invert it; slowly bring it back to its former position—the idea is to fill the agglutination tube inside of the large tube; finally pour out enough of the urine until the top of the agglutination tube becomes visible; the latter must not contain any air bubble whatsoever. Fill another set of tubes with plain water, in which a little yeast has been emulsified, and use this as a control. Place both in the incubator at 37° to 40° C., until the next day, and then ascertain whether any bubble of gas has appeared at the top of the agglutination tube; if so, a fermentable sugar must have been present. This test must always be employed when the two other tests described furnish a doubtful result.

(3) *Test for Indican as Follows.*—To about 5 c.c. of urine add an equal amount of Obermeyer's reagent and 1 c.c. of chloroform; close the tube with your thumb and invert a dozen times. In the presence of a normal quantity of indican the chloroform will either show no color at all or a light blue. A dark blue indicates an increase.

(4) *Test for Diacetic Acid as Follows.*—To about 5 c.c. of urine add 10 drops of a 10 per cent. chloride of iron solution. If a Burgundy color develops the reaction may be due either to diacetic acid or to the presence of a drug—salicylic acid or one of its derivatives. To differentiate between the two dilute 5 c.c. of urine with an equal volume of water, boil down to the 5 c.c. mark and then add the chloride of iron. If the color now does not appear the original reaction was due to diacetic acid.

(5) *Test for Acetone.*—Take 5 c.c. or more of urine in a test-tube; add a few crystals of sodium nitroprusside; shake until the latter has dissolved; alkalinize strongly with 20 to 30 per cent. sodium hydrate solution; then allow glacial acetic acid to slowly flow down the side of the tube. The appearance of a Burgundy color at the zone of contact indicates the presence of acetone.

(6) *Test for Chlorides.*—If albumin is present this must first be removed by boiling, after acidifying with a few drops of 5 per cent. acetic acid; filter; to about 10 c.c. of the filtrate add approximately

1 c.c. of concentrated nitric acid and then a few cubic centimeters of a 5 per cent. solution of silver nitrate. If a heavy flocculent precipitate develops the chlorides are present in normal amount. If an opalescence only results they may be regarded as absent.

(7) *Test for Bile Pigment.*—In the presence of bile pigment a greenish ring will be seen to develop at the zone of contact between the urine and the nitric acid when the latter test is applied, as in the examination for albumin. When in doubt overlay about 5 c.c. of urine with 2 c.c. of tincture of iodin that has been diluted 1 to 10 with alcohol. At the zone of contact an emerald-green ring will develop. Apply this test also to urine C.

Write up your findings in A, B, and C in the form of a formal report or in the form of a letter directed to a fellow doctor.

Answer the Following Questions in Writing.—What does the specific gravity of the urine indicate? What is the normal volume for twenty-four hours? What is meant (*a*) by oliguria, (*b*) anuria, (*c*) polyuria? What does a low specific gravity indicate? Under what pathological conditions would you expect a low specific gravity and under what conditions a high one? What would a greenish-brownish color of the urine suggest? What a red or reddish-brownish color? Under what conditions would the reaction of the urine be alkaline? What would an alkalinity due to volatile alkali indicate? How would you determine whether the alkalinity in a given case was due to fixed or volatile alkali? Under what pathological conditions are the chlorides absent? Under what conditions would bile appear in the urine? Under what conditions would you expect acetone and diacetic acid in the urine? What are the albumins that are ordinarily met with in the urine? What is meant by Bence-Jones albumin? Enumerate the conditions under which albumin may appear in the urine? What is meant by alimentary glycosuria? How would you differentiate between the latter and true diabetes—arrange your statements in tabulary form? What does an increase in the amount of indican indicate?

Reading.—

Lesson 37.—*Material, Apparatus and Reagents Required.*—Furnish every six students with 150 c.c. each of a normal, of an albuminous and of a diabetic urine—marked correspondingly—; note the specific gravity and the twenty-four hour amount of each on the beakers. All three should have been preserved with chloroform. Also supply every six men with 150 c.c. of Esbach's reagent; 500 c.c. of

Benedict's solution for the quantitative estimation of sugar; 200 grams of crystallized sodium carbonate and a few grams of talcum powder; 1 oz. of a saturated solution of potassium chromate and 300 c.c. of a $\frac{N}{10}$ solution of silver nitrate; 1 oz. of 5 per cent. acetic acid; 1000 c.c. of distilled water.

Supply every man individually with the following apparatus: One 100 c.c. cylinder; one 150 c.c. beaker; one urinometer; one 50 c.c. Mohr's burette, graduated in tenths, with stand; one evaporating dish—150 c.c. capacity; one stirring rod with rubber tip; one tripod with wire gauze; one 5 c.c. pipette; one 10 c.c. pipette; one 25 c.c. pipette; one Esbach albuminimeter with stopper and stand.

Directions to the Student.—(A) Fill your albuminimeter with the albuminous urine to the mark U—lower meniscus at U—; add Esbach's reagent to R—lower meniscus; close the tube with its stopper; invert it a number of times; then set it aside in its stand —vertically—for twenty-four hours. (B) Determine the quantity of sugar present in the diabetic urine as follows: If the specific gravity of the urine is 1030, dilute five times; if it is higher, dilute ten times, *i. e.*, 1 in 5 or 1 in 10. Calculate out the requisite amount of urine to make up 50 c.c. of its dilution.[1] Use the 10 c.c. pipette and dilute with water in the 100 c.c. cylinder. Fill your burette with the diluted urine; see to it that there are no bubbles in the rubber cuff and that it is full to the tip. Place 25 c.c. of Benedict's solution into your evaporating dish, together with 10 to 20 grams of sodium carbonate—roughly half a teaspoonful—and a pinch of talcum powder; arrange the evaporating dish on the tripod, underneath the tip of the burette which has been charged with the urine, as just directed. Note your reading and then slowly heat the mixture in the evaporating dish to the boiling-point. While the solution is gently boiling, run in the urine, rather rapidly, stirring all the time, until every trace of blue has disappeared. Water may be added during the process, if the fluid becomes too concentrated. Take your second reading and ascertain the number of cubic centimeters of the diluted urine that were necessary to bring about decolorization of the Benedict solution. Make a second titration and average your results. The titer of the Benedict solution is such that 25 c.c. will be reduced by 0.05 gram of dextrose. Calculate the percentage present in the urine under examination, and from this the total

[1] Before diluting the urine, boil it in a beaker for a few moments to remove traces of chloroform, which was used as a preservative and which itself reduces cupric salts.

amount voided in twenty-four hours. (C) Determine the quantity of chlorides present in the normal specimen of urine as follows: Charge your burette, after carefully washing it out, first with tap and then with distilled water, with from 20 to 30 c.c. of $\frac{N}{10}$ silver nitrate solution—fill to the tip and eliminate air bubbles from cuff. With a pipette measure off 5 c.c. of the urine; place this in a beaker; add 50 to 75 c.c. of distilled water and 10 to 15 drops of the solution of potassium chromate as indicator; take your reading and, while stirring, allow the silver nitrate solution to run into the diluted urine until a reddish color appears and persists; then take your second reading; the difference represents the number of cubic centimeters of the silver solution which were necessary to precipitate the chlorides contained in 5 c.c. of urine; 1 c.c. of the silver solution will precipitate the chlorine contained in 0.00585 gram of sodium chloride. Calculate the percentage of NaCl contained in the urine examined as well as the amount eliminated in twenty-four hours. (D) Determine the chlorides present in the albuminous urine. To this end take about 10 c.c. of urine; acidify with 5 to 10 drops of 5 per cent. acetic acid; boil and filter—to remove the albumin; then proceed with the filtrate as directed sub. C. Submit all your quantitative results in the form of a letter, addressed to a colleague. Give the total quantity of urine, specific gravity, percentage and total amount of albumin, sugar and chlorides voided in twenty-four hours.

Answer the Following Questions in Writing.—What is the normal output of chlorides, of total nitrogen, of urea, of uric acid and of creatinin in twenty-four hours? What is the normal two-hour response to Mosenthal's nephritic test-meal (*a*) as regards the quantity of urine voided; (*b*) as regards the specific gravity; (*c*) as regards the elimination of sodium chloride, and (*d*) as regards the elimination of the total nitrogen? Give findings in tabulary form. In what respect does the response of the nephritic differ from that of the normal individual? What is meant by the functional renal tests? How is Ambard's coefficient obtained? What information may be derived from the lactose test and how is the latter conducted?

Reading.—

Lesson 38.—*Material, Apparatus and Reagents Required.*—(A) *For the Performance of the Permeation Test.*—Administer the phenolsulphonephthalein test to a number of normal and nephritic individuals and have the urine collected from each, at the end of one hour, and after two hours, following the injection of the drug; pre-

serve the specimens with chloroform, if the test is given the day before the exercise, and mark the bottles with the name of the patient as well as "after one hour" and "after two hours." Furnish every six men with a normal set and one from a case of nephritis.

Supply every six men with the following: one 250 c.c. graduate; 100 c.c. of a 10 per cent. solution of sodium hydroxide; one liter of distilled water. Have available as many Sahli colorimetric tubes and stands with the necessary standard color tubes (I, II and III) of phenolsulphonephthalein (as furnished by E. Leitz & Co.) as possible, together with a corresponding number of test-tubes, marked at 2 c.c., 5 c.c., and 10 c.c. respectively; a 2 c.c. pipette and an ordinary dropping pipette provided with a rubber nipple.

Directions to the Student.—Let one man of each group act as "preparateur" for his group. His duty shall be to measure the quantity of urine voided by each case under examination, taking the one-hour and two-hour specimen of each separately. He shall note the quantity on each bottle or glass, and shall then add 10 c.c. of a 10 per cent. sodium hydroxide solution, and dilute each specimen with water up to the 200 c.c. mark. Note that the color of the urine turns reddish.

Every student of the group is then to withdraw 2 c.c. of this colored fluid, which is received in the graduated test-tube and examined as follows: dilute with distilled water to the 10 c.c. mark; mix well. Transfer enough of this diluted fluid to the colorimetric tube to reach the mark 50. Now compare the color with the standard I and II. If it is darker than I it is diluted, drop by drop, with water until the colors match, the percentage being read off the same as in estimating the hemoglobin. If tube II is used the result must be divided by 2. Should the color be too light after the urine has been diluted to 10 c.c. prepare another specimen and dilute only to 5 c.c. Examine this as just directed, but divide the final result by 4. If the urine contains very small amounts of the excreted phenolsulphonephthalein, then after it has been made up to 200 c.c. it should be poured directly into the calibrated tube up to the mark 50. The color must then be compared with the special color tube III, in which the yellowish tint of the less diluted urine has been compensated. Water is now added, until the colors match, when the reading obtained is divided by 20. Write up your findings in the form of a report addressed to a colleague.

Answer the Following Questions in Writing.—How soon after the injection should the phenolsulphonephthalein appear in the urine

under normal conditions? How large a percentage of the injected material should be eliminated during the first hour and how large a percentage during the second hour? What would your findings be if the integrity of the kidneys were seriously impaired? What are the indications for the use of the test?

Reading.—

(B) *The study of acidosis by the determination of the tension of the alveolar carbon dioxide.* Furnish every six men with a Marriott outfit for the determination of the alveolar carbon dioxide tension; have available for every individual man a calcium chloride tube or similar contrivance, moderately tightly packed with cotton (discard the cotton after use and sterilize the tube by boiling or in the hot-air sterilizer).

Two students should work together, one as examiner, the other as subject.

First go over the contents of the box; then proceed as follows: By means of an atomizer bulb fill the rubber bag of the outfit a little less than half-full with air, and clamp off the rubber tube. Interpose a *fresh* cotton filter tube between the bag and the mouth-piece. The subject, *at the end of an expiration,* takes the mouth-piece into his mouth while the examiner holds the subject's nose closed; the individual now breathes back and forth into the bag, four times in twenty seconds, the examiner marking the time. The tube is then immediately clamped off and the analysis of the respired air started within three minutes, as carbon dioxide escapes through rubber. To this end the test-tube accompanying the instrument is charged with 2 or 3 c.c. of the accompanying solution of bicarbonate of soda, colored with phenolsulphonephthalein, when air from the bag is forced through (by means of the finely drawn out glass tube), until no further change in color takes place. The tube is then stoppered and the color compared with the standards in the little stand. The number on the standard tube which is matched indicates the corresponding tension. Make duplicate determinations until two successive ones do not differ by more than 2 mm.

Answer the Following Questions in Writing.—What is meant by acidosis? Under what pathological conditions is it encountered? Name the acids which are concerned in its production? In what manner does their production lead to deleterious results? What is meant by the reserve alkali of the blood? What is the normal hydrogen ion concentration of the blood? What is found in acidosis?

What causes this change? Why may the carbon dioxide tension of the alveolar air serve as an index of the existence of acidosis and its extent? What is the normal tension? In what direction is it changed in acidosis? What is meant by the ammonia coefficient of the urine? What is it normally? In what direction does it tend in acidosis? Why may the ammonia coefficient serve as an index of the existence of acidosis and its degree? What is meant by alkalinosis? Under what condition does it occur? What is the mother substance of acetone in cases of acidosis? Write the chemical formula expressing its origin.

Reading.—

Lesson 39.—*Material Required.*—Have available for every six students a set of tubes containing as extensive a collection of urinary sediments or centrifugates as possible, each being suspended in a few cubic centimeters of urine or saline. It is desirable to collect large amounts of urine of each type and to preserve it with chloral. The sediment may then be allowed to form spontaneously; the supernatant fluid is pipetted or siphoned off, when the remaining sediment may be further concentrated as desired and finally distributed in tubes—one tube of each kind for every six men. Provide each tube with an ordinary pipette and label it as to contents. One set of tubes (*A*) should contain those non-organized constituents which might be encountered in an acid urine, such as (*a*) uric acid—various forms; (*b*) calcium oxalate—various forms; (*c*) primary calcium phosphate and (*d*) cystin. One tube (*e*) should contain those non-organized constituents which one would meet with in an alkaline urine—the alkalinity being due to fixed alkali—viz., basic phosphate of magnesium and calcium, ammonio-magnesium phosphate, calcium carbonate and neutral calcium phosphate. Another tube (*f*) should contain those non-organized constituents which we would meet with in an alkaline urine whose alkalinity is due to volatile alkali, viz., ammonium urate—various forms—in addition to the constituents of the preceding tube. (*B*) The next set of specimens should contain (*a*) mucous cylinders, (*b*) the various kinds of casts, (*c*) epithelial cells, (*d*) pus corpuscles, (*e*) red cells and red cell shadows (from a case of nephrolithiasis) and (*f*) spermatozoa. (*C*) A final tube should contain the concentrated morphological constituents of a case of renal tuberculosis, including the bacilli in question.

Reagents Required.—For every six men 1 oz. of a 1 per cent. aqueous solution of eosin; 4 oz. of Gabbett's carbol fuchsin solution; 4 oz. of Gabbett's acid methylene-blue solution; 1 oz. of a 10 per cent. acetic acid solution; 1 oz. of 30 per cent. hydrochloric acid; 1 oz. of 25 per cent. caustic soda solution; 1 oz. of ammonium hydroxide. Have available also staining trays, wash bottles and medicine droppers or capillary pipettes.

Directions to the Student.—Mount drops of the various sediments on slides, spread the material out and adjust a cover-glass over a portion of each specimen. Examine first with the low power—with the condenser thrown out, and using the flat mirror, so as to secure a subdued light; then with the middle power, making use of that portion of the smear that has been covered with the cover-glass—more light is then needed, to which end the convex mirror is substituted for the flat mirror. Now make careful drawings of your findings and append corresponding legends. Sub A–a, note that all uric acid crystals are colored—unless they are so thin that the quantity of coloring matter present is insufficient to be recognized by the eye. The key form is the rhomb or whetstone. Draw individual crystals and a group—color them with your pencil. Add a drop of 25 per cent. caustic soda—do the crystals dissolve? Sub A–b, note that the typical oxalate crystal, no matter how small, may always be recognized under the low power by the appearance of a dark surface side by side with a brightly illuminated surface—a black spot alongside of a high light. Examine with the middle power and note that this is due to the octahedral form of the crystals; note the highly refractive cross in the interior which comes out on focussing, and which in the larger specimens may even be seen with the low power. Allow a drop of acetic acid to flow under the cover-glass—the crystals do not dissolve; substitute a drop of hydrochloric acid—do they dissolve? Are the crystals colored? Sub A–c, note the prismatic form of the crystals; are they colored? Do they dissolve in acetic acid? Draw. Sub A–d, note the hexagonal form of the cystin platelets. Are they colored? are they soluble or insoluble in the following reagents—hydrochloric acid, acetic acid, ammonia? Draw. Sub A–e, note the acicular crystals of neutral calcium phosphate, the occurrence of basic phosphate of calcium and magnesium in granular form or of the latter in the shape of large highly refractive plates, and the large prismatic crystals of ammonio-magnesium phosphate (triple phosphate) in the shape of the lid of a German coffin (coffin-lid crystals). All these are soluble in acetic acid—test

this. Draw. Sub A–f, note the dumb-bell shaped crystals of calcium carbonate, and the fact that they dissolve in acetic acid, with the evolution of gas bubbles. Draw. Ammonium urate occurs in the form of brownish spherical bodies of variable size, which are sometimes composed of delicate needles, while at others they are amorphous and sometimes beset with prismatic spicules—thorn-apple forms. Ammonium urate is soluble in acetic acid and hydrochloric acid; subsequently rhombic crystals of uric acid separate out. Sub B–a, note the irregular, longitudinally striated, band-like structure of the mucous cylinders. Draw. Are they soluble in dilute acetic acid? Sub B–b, note the irregular, polyhedral or fusiform and rolled-up appearance of the so-called vaginal epithelial cells and the large, flat, roughly quadrangular cells from the urethra. Observe the large round and oval, sharply contoured cells from the bladder. Draw. Sub B–c, note the parallel outline and solid, sausage-shaped appearance of the true casts. Note how essential it is to have a subdued light, by turning on full light, when it will be seen that the hyaline casts have become invisible. Study the character of the "impedimenta" which some of the casts carry, such as black amorphous granules (finely granular and coarsely granular casts), brown granules (brown granular casts), red cells (blood casts), leukocytes (leukocytic and pus casts—when leukocytes are abundant), renal epithelial cells (epithelial casts), fat globules (fatty casts). Draw all these appearances. Search for an epithelial cast; note the size and oval form of the cells, the granular character of the protoplasm and the presence of a single nucleus; next search for free cells of the same type. Draw. Add a drop or two of eosin solution to your specimen and note the manner in which the casts take the dye; the hyaline casts and the hyaline matrix of the compound hyaline casts is colored pink; the granules turn a deeper color. Search for large coarse casts presenting a vermilion color—many of these in the unstained specimen appear yellowish—waxy casts. Draw. Add a few drops of dilute acetic acid—what happens to the hyaline casts? Sub B–d, note the appearance of the leukocytes—pus corpuscles—under the low power; add a drop of acetic acid, and examine with the middle power—the multiple nuclei are now well shown. Draw. Sub B–e, note particularly the blood shadows, *i. e.*, red cells which have lost or are losing their hemoglobin. Learn to recognize the intact red cells with the low power, under which they appear as mere rings. Compare with the appearance of the leukocyte—the one a ring, the other a solid. Draw.

Sub B–f, note the appearance of the spermatozoa; learn to recognize them with the low power; search for corpora amylacea. Draw. Sub C, finally, prepare fairly thick smears of the centrifugate from the case of renal tuberculosis. Examine while wet; note the presence of pus cells and red cells. Dry the specimen carefully by beating the flame of the Bunsen burner; then stain for tubercle bacilli, as directed in Lesson 19. Wash off; dry and examine carefully with the oil-immersion lens; search for bacilli lying singly and in clusters. Make a composite drawing in colors.

Answer the Following Questions in Writing.—What clinical significance attaches to the habitual passage of urine that shows a marked tendency to the spontaneous and abundant deposition of sediments composed of uric acid or oxalate of lime crystals? Does the appearance of an abundant sediment of crystals of a substance warrant the conclusion that the substance in question is being eliminated in increased amount? What significance attaches to the appearance of hyaline tube casts in the urine? What conclusion would you draw from the appearance of blood casts and epithelial casts and leukocytic casts? What is pyuria? Enumerate the conditions under which pus might appear in the urine? How would you determine its source? Under what conditions does blood appear in the urine? What urinary findings would lead you to make an examination for tubercle bacilli? Should your search for the latter be negative and there be a clinical suspicion of the existence of renal tuberculosis nevertheless, what would your course of procedure be to prove its existence? What microörganism is most frequently associated with pyuria in women? How would you examine for its presence?

Reading.—